4

Introduction to Candida
Systemic Candidiasis

JUDITH E. DOMER and ROBERT I. LEHRER

1. INTRODUCTION TO CANDIDA

1.1. Description of the Disease

Candidiasis is the prototypic opportunistic fungal disease. *Candida* spp. reside most commonly in the gastrointestinal tract[1,2] and vagina, where they are normally held in check by as-yet incompletely defined local factors that include competition with the resident bacterial flora and innate effectors of nonspecific mucosal resistance.[3,4] Infrequently, but under a wide variety of clinical circumstances, these normally inconspicuous commensals can multiply on or invade through the mucosal surface to initiate local or systemic disease. Various immunologic and nonimmunologic conditions can predispose to such opportunistic infections.[5] Among the former are certain malignancies[6–8] and the use of cytotoxic or immunosuppressive therapy.[9,10] Nonimmunologic predisposing factors include diabetes,[11] trauma,[12] pregnancy,[13] antibiotic therapy,[7] and hyperalimentation.[14] Not infrequently, such predisposing factors occur in combination.

Significant risk factors for nosocomial candidiasis in patients with cancer include previous surgery, neutropenia, central catheterization, chemotherapy, specific antibiotic treatment, and peripheral cultures positive for

JUDITH E. DOMER • Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, Louisiana 70112. ROBERT I. LEHRER • Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024.

Fungal Infections and Immune Responses, edited by Juneann W. Murphy *et al.* Plenum Press, New York, 1993.

Candida spp.[15] Additional risk factors may include the presence of a bladder catheter, treatment with two or more antibiotics, azotemia, transfer from another hospital, diarrhea, and candiduria.[16] The potential roles of dietary, mechanical or iatrogenic factors have been reviewed by Odds,[17] and the clinical and microbiological aspects of nosocomial candidiasis have been reviewed by Pfaller.[18]

Although the syndromes of candidiasis can be broadly categorized into cutaneous, mucocutaneous, and systemic forms, more specialized classifications based on the predominant site(s) of infection are useful clinically. Systemic infections can include prosthetic valve disease, endophthalmitis, hepatosplenic candidiasis, peritonitis, endocarditis, meningitis, arthritis, and osteomyelitis, whereas mucocutaneous infections can include esophagitis, vulvovaginitis, cystitis, oral thrush, and denture stomatitis.[17,19]

In recent years, mucocutaneous candidiasis has garnered much attention because an especially noxious form that affects the mouth, tongue, and esophagus is very common among patients infected by human immunodeficiency virus (HIV).[20–22] In general, it appears that granulocytopenia or defective phagocyte function promotes systemic disease, whereas reduced T-cell/macrophage function promotes mucocutaneous disease. As the immunology of cutaneous and mucocutaneous disease is covered in Chapter 5, this chapter will center on systemic candidiasis. Although results obtained from animal models are featured prominently in this chapter, where possible, we will highlight results obtained with human materials. An excellent review of the animal models used to study various forms of candidiasis was published by Guentzel *et al.*[23]

1.2. Etiology

There are almost 200 recognized species in the genus *Candida*,[24] including those organisms formerly classified as *Torulopsis* spp., but only a few species are part of the normal flora and/or regularly cause human disease. The two most common pathogenic *Candida* spp. are *C. albicans* and *C. tropicalis*. Although precise figures are difficult to obtain, *C. albicans* appears to be responsible for approximately 60–75% of all candidal disease, *C. tropicalis* for 15–25%, *C. glabrata* for 10–15%, *C. parapsilosis* for 5–15% and *C. krusei* for about 2%.[6,25] *C. tropicalis* appears to be less pathogenic than *C. albicans* under most circumstances, but disease caused by *C. tropicalis* has become more prevalent, especially in individuals with hematologic malignancies and those undergoing bone marrow transplantation.[26,27] Based on their histopathologic observations of the GI tracts of individuals with *C. tropicalis* and *C. albicans* infections, Walsh and Merz[28] proposed that *C. tropicalis* was more enteroinvasive than *C. albicans* in susceptible hosts. Howlett[29] reported that *Candida* spp. differ in their ability to penetrate keratinized rat tongue mucosa (*C. albicans* > *C. tropicalis* and *C. krusei* > *C. parapsilosis* and *C. guilliermondii*),

and a more recent study suggests that clinical isolates of *C. albicans* differ in their pathogenicity for dorsal rat tongue mucosa.[30] The ability of *C. albicans* to form hyphae is believed to be important for colonization and invasion of the oral and vaginal mucosa.[31,32] Species with less pathogenic potential, e.g., *C. parapsilosis*, *C. krusei* and *C. guilliermondii*, are often associated with a more restricted range of clinical conditions. For example, *C. parapsilosis* is found in paronychia, endocarditis, endophthalmitis and otitis media; *C. guilliermondii* causes endocarditis, cutaneous candidiasis and onychomycosis; and *C. krusei* occasionally causes endocarditis.[18,19,33] Since the three organisms mentioned last are more commonly found among the flora of skin than in the gastrointestinal tract,[19] this ecological niche might position them to initiate the clinical syndromes mentioned previously. *C. lusitaniae*, an organism which the diagnostic laboratory can easily confuse with *C. tropicalis*, and *C. parapsilosis* are also being identified more frequently as a cause of disease in immunosuppressed individuals.[34] Infections due to *C. lusitaniae* and *C. guilliermondii*, *Candida* spp. of low intrinsic virulence, can be difficult management problems since either organism may be resistant to polyene antibiotics.[35,36]

1.3. Epidemiology

Although various species of *Candida* can be isolated from lower animals and from numerous environmental sources, summarized in Odds,[17] most human candidal disease was considered to be of endogenous origin until a few years ago. It has been known for some time that there are two primary serotypes of *C. albicans*, A and B.[37] Since all *C. albicans* strains have identical sugar assimilation and fermentation patterns when tested with the usual limited battery of substrates, and since disease was considered to be endogenous, little effort was expended in attempting to determine intraspecific strain differences until recently. During the last decade, at least eight typing methods that are potentially applicable to the epidemiology of *C. albicans* infections have been developed. The methods include biotyping,[38,39] resistotyping,[40] sensitivity to killer yeasts,[41] extracellular enzyme production,[42] immunoblotting,[43] morphotyping,[44] DNA restriction fragment analysis,[45,46] and electrophoretic karyotyping.[47,48]

Such methods have been used to investigate outbreaks,[49,50] to determine the dominance of a strain type in a clinical syndrome,[51,52] or simply to survey the biotypes predominant in an institutional or geographic environment.[53–55] In two of these studies,[53,55] isolates of *C. albicans* from different anatomic sites in the same patient appeared to be the same, although Soll *et al.*[56] isolated multiple strains and species of *Candida* from a single patient from different anatomic sites at different times during the course of disease. The studies to date have been limited, but some candidal infections may prove to be acquired from exogenous (possibly nosocomial) sources rather than from the patient's own endogenous yeast-cell flora.[54]

2. SYSTEMIC CANDIDIASIS

2.1. Innate Immunity

2.1.1. Nutritional Control Mechanisms

Although *C. albicans* requires biotin for growth,[57,58] its other nutritional needs can be satisfied by simple, fully-defined media that contain any of a wide range of carbon sources; ammonium, sulfate and phosphate salts; and trace elements. Notwithstanding this metabolic versatility, some of the innate mechanisms responsible for inhibiting *Candida* spp. proliferation *in vivo* may operate on the level of substrate deprivation or sequestration. The best established of these mechanisms involves the sequestration of iron by two host-derived iron-binding proteins, transferrin and lactoferrin. Indeed, the observations that normal human serum inhibited the growth of *C. albicans in vitro* and that this effect was reversed by addition of iron were directly responsible for the discovery of transferrin,[59] the major iron-binding protein of serum. The specific granules of neutrophils contain large amounts of an iron-binding protein, apolactoferrin, that is structurally homologous to transferrin.[60–62] Not surprisingly, apolactoferrin has also been shown to be candidastatic by virtue of its ability to bind iron.[63] Recent evidence[64–66] suggests that *in vitro* and *in vivo* growth restriction of *C. albicans* may also be mediated by a calcium and/or zinc-binding protein that has been found in the cytoplasm of neutrophils and certain epithelial cells by a number of workers. These studies have been reviewed by Steinbakk *et al.*[66] Ongoing work by Wang and Lehrer (unpublished data) suggests that competition for biotin between *C. albicans* and various bacteria requiring biotin, including oral streptococci, may play a role in limiting the population density of *C. albicans* in the mouth. An increased local population of *C. albicans* occurs in the oral cavity of many diabetics and may be attributable to the higher concentrations of glucose in oral secretions from hyperglycemic individuals.[67] Other host factors that may regulate the growth of oral *C. albicans* have been reviewed by Epstein *et al.*[68]

The factors responsible for maintaining relatively low concentrations of *C. albicans* within the gastrointestinal tract are understood only in part, and include competition with other members of the intestinal flora and host immune factors. The areas of colonization are highly selective and may be related to the surface characteristics of the affected epithelium and its keratin.[69] In rats reared conventionally, the administration of broad-spectrum antibiotics[70] and corticosteroids[71] both predispose to long lasting intestinal carriage of *C. albicans*. Cantorna and Balish[72] examined the ability of *C. albicans* to colonize the gastrointestinal tract of germfree mice. Doubly immunodeficient beige and athymic (bg/bg and nu/nu) animals developed extensive yeast and hyphal infections of the palate, tongue, esophagus, and stomach, whereas singly

immunodeficient beige or athymic mice and their normal nu/+, bg/+ littermates developed only moderate mucosal infections. The doubly immunodeficient mice later also developed progressive systemic infections caused by the *C. albicans*. The possibility that the small intestine might contain and secrete antifungal components that might affect *Candida* spp. population density has not been examined systematically. It warrants attention, however, since murine Paneth cells, which line the base of the small intestine's numerous crypts, contain large amounts of mRNA for cryptdin,[73] a member of a family of peptides (defensins) many of whose members are strongly candidacidal.[74]

2.1.2. Intravascular Access and Clearance

Clinical disseminated (systemic) candidiasis results when *C. albicans* enters the vascular system in numbers that exceed the ability of host defense mechanisms to clear them and prevent their subsequent growth in the tissues. *C. albicans* probably most often gains entry to the vascular system from its reservoir in the gastrointestinal tract, but the organisms may also be introduced into the circulation from the skin via indwelling intravenous lines or by injection of contaminated materials. In a historic experiment that is not likely to be repeated soon, Krause *et al.*[75] administered a large number of viable *C. albicans* blastospores intragastrically to a normal human subject. Some of the fungi appeared in the circulating blood within 30 minutes, evidently having traversed the epithelial barriers of the normal human gastrointestinal tract to do so. Sass *et al.*[76] have provided evidence that the transgastrointestinal migration of particles, such as latex spheres, fungal spores, and pollen grains, is mediated by M cells in Peyer's patches and that subsequent intravascular access is provided via the abdominal lymphatics. Since even an intact gastrointestinal tract evidently permits some intraluminal *C. albicans* yeast cells to gain access to the blood, intestines damaged by disease, drugs, or trauma are likely to be even more pervious. Since bloodstream seeding by intestinal *C. albicans* undoubtedly is initiated by fungal organisms adherent to the epithelial surface, factors (e.g., broad spectrum antibiotics) that enhance *Candida* spp. growth by decreasing microbial competition for nutrients or anchorage sites probably increase the relative likelihood of hematogenous seeding.

Clearance of intravenously injected organisms from rabbit blood shows biphasic kinetics. Approximately 99% of the organisms leave the blood within 10 minutes, followed by a longer period of low-grade fungemia. Depending on the route of injection, different organs predominate in clearance. After peripheral venous injections, lungs take up 45% of the total inoculum; after mesenteric vein injection, liver takes up almost 60%.[77] Mice injected with live, but not formalin-killed, *C. albicans* evidenced accelerated reticuloendothelial clearance of carbon particles.[78]

2.1.3. Polymorphonuclear Leukocytes

2.1.3a. Protective Role of Polymorphonuclear Leukocytes in Vivo. The polymorphonuclear leukocytes (neutrophils, PMNL) are extremely important in defense against systemic infection by *C. albicans*, as evidenced by the fact that a) neutropenic patients are highly susceptible to the development of candidiasis,[79,80] b) the disappearance of organisms from tissue parallels the appearance of PMNL,[81] and c) patients with leukocyte defects, such as those seen in hereditary or acquired myeloperoxidase (MPO) deficiency or chronic granulomatous disease (CGD), may experience fungal infections caused by *Candida* spp. and other organisms. (MPO is one component of the oxidative pathways involved in killing microorganisms within phagocytic cells. It is discussed in more detail in Section 2.1.3c.) The importance of the leukocyte, whose numbers may have been reduced by bone marrow suppressive disease or therapy, has been shown in animal models where granulocyte transfusions resulted in partial reversal of the effects of leukopenia.[82,83] For example, although normal dogs tolerate 10^7 *C. albicans* organisms administered intravenously, widespread disseminated candidiasis results when only 10^6 organisms are given to leukopenic animals. Granulocyte transfusions to the immunosuppressed dogs, however, afforded substantial protection against the fungus.[82] Studies with diffusion chambers placed intraperitoneally in mice have also provided evidence for the importance of phagocytes in clearing sites of *C. albicans in vivo*.[84]

Hereditary MPO deficiency, usually discovered after the routine use of certain automated blood count analyzers, appears to be a common but infrequently diagnosed condition. In a population of 60,000 patients tested at Stamford Hospital in Stamford, Connecticut over 40 months, 28 MPO-deficient persons were identified. Half of these had completely MPO-deficient PMNL and the others had partial deficiencies. Most MPO-deficient patients had no clinical sequelae, but several had experienced serious systemic infections caused by *Candida* spp. or other organisms. The most serious of these occurred in a 30-year-old woman who also had insulin-dependent diabetes mellitus and developed fatal pneumonia caused by *C. albicans* and *C. parapsilosis*. Overall, 4 of the first 29 reported individuals with complete (homozygous) hereditary MPO deficiency had systemic candidal infections.[85–89] Three of these persons had coexisting diabetes mellitus, which may also have contributed to their susceptibility to systemic candidiasis. If the frequency of homozygous hereditary MPO deficiency is as high as suggested by the Stamford Hospital study (14/60,000 = 0.00023) then, assuming an autosomal recessive pattern of inheritance, it can be calculated that the heterozygous carrier state is likely to exist in approximately 1.5% of the population. Although PMNL from subjects that were heterozygous for hereditary MPO deficiency contained decreased levels of MPO, their ability to kill ingested *C. albicans* was intact.[85] Consequently, it seems unlikely that partial

(heterozygous) MPO deficiency will prove to be a significant risk factor for systemic candidiasis in humans.

The association of acquired MPO deficiency with leukemic and preleukemic states is now well recognized.[90–92] In one such patient studied at UCLA, 99.2% of blood PMNL were completely MPO deficient, and the cells showed greatly impaired killing of ingested *C. albicans*, *C. tropicalis* and *S. aureus in vitro*.[93] The patient succumbed to fungal infections caused by *A. fumigatus* and *C. albicans*. Although PMNL from patients with hereditary complete MPO deficiency can phagocytize the organisms normally, they are greatly impaired in their ability to kill *C. albicans* and certain other *Candida* species, including *C. tropicalis*, *C. stellatoidea*, and *C. krusei*.[85,87,94] MPO-deficient PMNL also killed many, but not all, bacteria at a reduced rate.[86,95] Although MPO appears to be essential for effective killing of *C. albicans* by human PMNL and mononuclear (MN) cells,[85,96,97] other *Candida* species such as *C. parapsilosis*, *C. pseudotropicalis*, and *C. krusei* were killed effectively by MPO-deficient PMNL.[94] Certain of these (*C. parapsilosis* and *C. pseudotropicalis*) are also killed normally by PMNL from subjects with CGD.[94] It can be concluded from these observations that *C. albicans* is relatively resistant to human PMNL and that its eradication requires both MPO and hydrogen peroxide (H_2O_2). Other *Candida* species are killed effectively by PMNL mechanisms that require neither MPO nor H_2O_2. These nonoxidative mechanisms appear to be relatively ineffective in killing *C. albicans*, although some of them might contribute to candidastasis.

The effects of a number of additional underlying illnesses and their treatments on the candidacidal function of PMNL have been examined as well. Patients with neoplasms are predisposed to systemic candidal infections especially while receiving immunosuppressive therapy. PMNL from patients with acute leukemias are generally able to ingest *C. albicans* normally but frequently manifest decreased candidacidal activity.[98–100] Similar results have been described in patients with large burns.[101] PMNL from patients with chronic granulocytic leukemia have been reported to kill *C. albicans*[100] but not *C. guilliermondii*[102] normally. Subjects with myeloid metaplasia, polycythemia vera, untreated metastatic solid tumors, Hodgkin's disease, and other lymphomas had PMNL with normal candidacidal activity for *C. albicans*. Patients receiving chemotherapy or radiation therapy for these conditions often had PMNL with diminished candidacidal activity.[99] Another study using *C. guilliermondii* to study PMNL from patients with various leukemias and lymphomas noted the frequent occurrence (12 of 24 patients) of candidacidal defects, often corrected *in vitro* by addition of levamisole.[103] The PMNL of patients with AIDS-related complex (ARC) and AIDS associated with deep candidiasis were reported to have a normal ability to kill *C. albicans*.[104]

Although neonates are generally considered to be relatively immuno-compromised hosts and were reported to have PMNL with decreased candidacidal activity,[105] this finding has not been confirmed.[106,107] Malnourished

children, known to harbor large concentrations of *Candida* spp. in their upper intestinal tracts, were found to have PMNL with significantly decreased candidacidal activity.[108] The PMNL of selenium-deficient rats were reported to display impaired *in vitro* fungicidal activity for *C. albicans*.[109] Hyperosmolar conditions decreased ingestion and killing of *C. albicans* by human PMNL *in vitro*.[110] Normal phagocytosis but decreased cidal activity against *C. albicans* has been reported in PMNL obtained from children with thalassaemia.[111] Amphotericin B-resistant *C. tropicalis* developed in a patient whose PMNL displayed diminished candidacidal activity against both *C. tropicalis* and *C. albicans*, especially when tested in autologous serum.[112]

In addition to their fungicidal activities, there is some evidence that PMNL may protect some tissues from destruction by the fungus as well. Edwards *et al.*,[113] using an *in vitro* assay involving the development of a monolayer of endothelial cells,[114] noted that the endothelial cells were protected from the harmful effects of candidal hyphae if PMNL were present. The mechanism(s) has not been delineated, but PMNL were attracted to the sites of hyphal attachment and spread over the hyphal elements, both within and beneath the endothelial cells.

In the healthy host, there is no shortage of PMNL to fend off *Candida* spp. PMNL are the most abundant phagocytes of circulating blood. In man, they are normally present at a concentration of approximately $2.5–5.0 \times 10^6$/ml of blood, but this can increase several-fold in response to infection. Circulating mature PMNL are short-lived cells, $T_{1/2} = 6–7$ hours, with a minimal capacity for new protein synthesis. MN are only about 10% as numerous as PMNL in the peripheral blood, but after entering tissues, they undergo further differentiation which enables them to survive for days or weeks as macrophages.

2.1.3b. Interactions between Candida spp. and PMNL in Vitro. Although animal leukocytes are often used as surrogates for their human counterparts, the validity of this practice is more an article of faith than of science. Fortunately, the availability of human PMNL and MN has allowed their interactions with *Candida* spp. to be studied directly *in vitro*. Human blood is fungicidal for *C. albicans*, killing both blastoconidia and organisms bearing pseudohyphae up to 200 mm in length.[115] The candidacidal activity of blood can be attributed directly to its leukocytes, since neither plasma nor serum alone kills *C. albicans*.[116,117]

The relationship between *C. albicans* and the phagocytic process itself and associated events, such as chemotaxis, are incompletely understood, but some data have been gathered on chemotaxis and the opsonic requirements for phagocytosis of *C. albicans*. Viable or heat-killed *C. albicans* were chemotactic for human PMNL,[118] as was mannan, a major component of the candidal cell wall.[119,120] Intact cells and purified mannan activated the alternative pathway of complement,[118,121] suggesting that *Candida*-induced chemotaxis could result from complement activation. Cutler[122] described a

soluble factor from seven of eight isolates of *C. albicans* that was chemotactic for guinea pig PMNL in the absence of serum, a condition that precluded complement activation.

The role of complement in uptake and subsequent killing of *C. albicans* is somewhat controversial. Most studies have shown that complement enhances phagocytosis and suggested that both classical and alternative systems are involved.[123–129] While there is agreement that serum complement factors facilitate phagocytosis[85,130–132] and that intracellular killing of the organism is not influenced by the presence or absence of specific antibody,[123,127,132] there is disagreement on specific aspects of the role of C3. Kernbaum[126] reported that phagocytosis and killing were normal in the absence of C3, Yamamura and Valdimarsson[124] reported that C3 was not necessary for ingestion of the organism but was necessary for killing, and Morrison and Cutler[127] reported that C3 was necessary for ingestion to occur. In one study,[133] the heat- stable opsonic factor of normal human serum was believed to be naturally occurring, low-level anti-*Candida* IgG. High antibody concentrations, typically present in serum from individuals with chronic mucocutaneous candidiasis or some patients with disseminated candidiasis, inhibited candidacidal activity by normal PMNL without significantly impairing phagocytic uptake.[134–136]

Although most research emphasis has centered on the opsonic properties of complement and/or antibody, other factors may also influence ingestion of *Candida* spp. Ginsburg *et al.*[131] reported that polycationic substances, such as nuclear histones, that accumulate in lesions after cell death and tissue destruction might function as efficient opsonins. Fleischmann *et al.*[137] reported that defensins, which are also polycationic peptides, were nonspecific opsonins that promoted the uptake of *C. albicans* and various bacteria by rabbit alveolar macrophages. The ability of defensins to opsonize *C. albicans* for uptake by PMNL has not been reported.

Several *in vivo* studies have provided evidence that complement participates in resistance to systemic candidiasis. Mice with various genetic defects involving complement were more susceptible to systemic challenge than those without such defects,[123,138,139] as were normal guinea pigs depleted of the alternative pathway of complement activation by treatment with cobra venom factor.[140] Since two of the mouse strains were deficient in C5, these data lend support to the *in vitro* work of Morelli and Rosenberg[123] on the importance of C5 in the phagocytic process.

Unlike most bacteria, only a portion of *C. albicans* yeast cells ingested by human PMNL are killed intracellularly during the first few hours. This was first shown with a quantitative vital staining (methylene blue) assay, which revealed that PMNL from normal human donors killed 29% ± 7.4% of ingested yeast phase *C. albicans* after 60 min.[116] These data have been confirmed by assay systems based on Giemsa staining,[141] isotope incorporation or chromium release,[142] and colony counting,[130,143] although the latter generally provide higher estimates of killing (approximately 50% after 60

minutes) by PMNL and MN. Serum, although required for efficient phagocytosis, did not facilitate killing of ingested organisms in one study[116] but did so in another.[144] The presence of PMNL substantially enhanced the ability of purified human MN to kill *C. albicans*.[96] The ability of human PMNL to kill ingested *C. albicans* was abolished when the incubations were performed under anaerobic conditions or in the presence of MPO inhibitors (cyanide or azide), although ingestion of the yeasts was not inhibited by these treatments.

It is clear that *Candida* spp. differ in their susceptibility to the fungicidal mechanisms of the PMNL. Yeast forms of *C. albicans*, *C. tropicalis*, and *C. viswanathii*, three of the more pathogenic species of *Candida*, were killed more slowly and at higher murine peritoneal (thioglycollate-induced) PMNL:target ratios than less pathogenic species such as *C. guilliermondii*, *C. krusei*, and *C. parapsilosis*.[145] Nevertheless, all *Candida* spp. were killed. It is also clear that both yeast and filamentous (hyphal and pseudohyphal) forms of *C. albicans* can be damaged by PMNL, although the extent of damage and the mechanics of the killing process may differ, in particular, with those filamentous forms too large to be ingested by the PMNL. Scherwitz and Martin[146] reported 90% phagocytosis of yeasts versus 10% phagocytosis of germinated forms; Schuit[147] reported 35% killing of yeasts versus 10% killing of pseudohyphae; and Cockayne and Odds[148] reported that yeasts, germinated forms and hyphae, were all ingested to a similar extent but that the filamentous forms were killed to a greater extent than the other two forms. Additionally, Schuit[147] noted that both ingestion and killing were more rapid when MN were used instead of PMNL. The inconsistencies between the data from Schuit[147] and Cockayne and Odds[148] emphasize the difficulties in comparing data when different assay conditions are used. Brummer *et al.*[149] compared the ability of murine peritoneal and circulating PMNL to kill *C. albicans* and reported that PMNL obtained after intraperitoneal placement of proteose peptone and caseinate did not differ significantly from peripheral blood PMNL in their ability to kill *C. albicans*, whereas cells recovered after thioglycollate instillations were defective.

Various antibiotics may affect the ability of phagocytes to ingest and kill *C. albicans*, an important observation when one considers that antibiotic treatment is one of the two most important predisposing factors to the development of systemic candidiasis, the other factor being neutropenia. Sulfonamides interfered with killing of ingested *C. albicans* by PMNL by inhibiting myeloperoxidase(MPO)-mediated reactions.[143] Penicillin, tetracycline, chloramphenicol, and gentamicin did not interfere with PMN candidacidal activity in one study,[150] although five aminoglycosides (including gentamicin) inhibited this function in another.[151] Ketoconazole and Sc-39304 were reported to enhance the ability of human PMNL to kill ingested *C. albicans*,[152] although the methods used may have assessed only those events transpiring in unsealed phagocytic vacuoles. Cilofungin (LY 12109) was reported to impair PMNL candidacidal activity by interfering with opsoniza-

tion and subsequent ingestion.[153] High levels of glucose and β-hydroxy butyrate were reported to inhibit the candidacidal activity of PMNL from diabetic, but not normal, subjects.[154] Amphotericin B has been reported to inhibit phagocytosis of *C. albicans* by PMNL.[155]

There have been relatively few ultrastructural observations of phagocytosis and killing of *C. albicans* by PMNL. Belcher *et al.*,[156] and Richardson and Smith,[157] working with human and murine PMNL respectively, observed the postphagocytic fusion of PMNL lysosomes with tight and loose phagosomes, as well as the presence of *C. albicans* cells with intact cell walls but disintegrating plasma membranes and intracellular structures. Shortly after the observations of Richardson and Smith[157] appeared, Cech and Lehrer[158] reported that *C. albicans* ingested by human PMNL were found in two types of phagolysosomes: "unsealed vacuoles," which maintained communication with the extracellular compartment and "sealed vacuoles," which did not. Approximately 40% of all PMNL vacuoles were unsealed, and only 15% of the *C. albicans* blastospores within them had been killed within 1 hour. In contrast, approximately 72% of *C. albicans* inside the sealed vacuoles had been killed. The sequestration of *C. albicans* in sealed and unsealed PMNL vacuoles was confirmed by Levitz *et al.*,[159] who also extended these observations to MN. Many *C. albicans* blastospores within unsealed vacuoles remained viable after their ingestion by PMNL. Perhaps leakage of potentially fungicidal molecules (e.g., MPO, defensins, H_2O_2, etc.) from unsealed vacuoles, or ingress of inhibitory ions and proteins from serum, or the combined effects of both processes contributed to the observed fungicidal activity.

2.1.3c. Oxidative Candidacidal Mechanisms of PMNL. The fungicidal mechanisms of human PMNL are of two general types: oxidative and nonoxidative. The former, which appear to be most important with respect to *C. albicans*,[85,116,141] depend on the neutrophil's ability to synthesize a group of potent oxidants, including H_2O_2, hydroxyl radicals, hypochlorous acids and chloramines. In normal PMNL, H_2O_2 is derived from the dismutation of superoxide anions, which are produced by an NADPH oxidase activated in PMNL after phagocytosis or stimulation by a variety of soluble mediators. The production of hypochlorous acid and chloramines by PMNL results from the ability of its MPO, an enzyme also found in MN, to catalyze the oxidation of chloride ion by H_2O_2.[160] MPO occurs in the primary (azurophil) granules of normal human PMNL and is released into their phagocytic vacuoles and extracellularly when PMNL are exposed to microbes, including *C. albicans*. Although MPO is present in bone marrow promonocytes and most blood MN, it is lost when these cells mature into macrophages. A distinctly different peroxidase that exists in human eosinophils can generate hypobromous acid from bromide ions and H_2O_2.[161] Human eosinophils can also kill ingested *C. albicans* by oxidative mechanisms.[162] Much evidence indicates that phagocytic normal human granulocytes produce hypochlorous acid[163,164] and that hypochlorite, chloramines, and cell-free MPO-H_2O_2-halide systems kill *Candida*

spp. and other fungi *in vitro*.[165–169] Blastoconidia from opaque phenotypes of *C. albicans* strain WO-1 were more susceptible to these oxidants than were white phenotypes.[170]

The mechanisms involved in intracellular killing of *C. albicans* by PMNL have been studied actively for many years. That oxidative mechanisms, specifically those mediated by MPO and H_2O_2, are very important, was first reported over twenty years ago.[85,116] The mechanisms of oxidant production and their significance for neutrophil-mediated antifungal activity have been clarified by studies of PMNL from patients with CGD and hereditary MPO deficiency. The PMNL of CGD patients are profoundly deficient in their ability to generate superoxide anion (O_2^-) and H_2O_2 via the NADPH oxidase pathway as a consequence of various distinct molecular lesions. Most cases of CGD show X-linked inheritance and deficient PMNL function that arises from abnormalities affecting the large subunit of cytochrome b_{558}, a critical component of the activated NADPH oxidase complex. Other forms of CGD are autosomal and result from abnormalities of cytosolic activators of NADPH oxidase or from abnormalities affecting the small subunit of cytochrome b_{558}.[171,172] In their review of 245 patients with CGD, Cohen *et al.*[173] reported that severe fungal infections, especially *Aspergillus* spp. pneumonia, occurred in 50 cases.

Since the infected host must deal with both yeast and hyphal forms in candidal lesions, a few investigators[146–148] have compared the interaction of PMN with the yeast and hyphal forms of *C. albicans*, but many more have examined exclusively the yeast form[85,116,123,124,130,141,147,149,156–158,174–177] or hyphal/pseudohyphal forms.[97,113,153,159,167,168,178–184] Unfortunately, comparisons of data generated from *in vitro* systems in different laboratories are complicated by the differences in their effector:target ratios, incubation times, methods for determination of ingestion and/or killing, animal and anatomic sources of effector cells, and incubation conditions—especially with respect to the presence and concentration of serum in the assay. Many such studies involving either PMNL or mononuclear phagocytes and the yeast form of *C. albicans* have been tabulated in another recent review.[5]

Diamond and his colleagues have provided detailed observations concerning the contribution of oxidative mechanisms to the ability of human PMNL and MN to kill *C. albicans* hyphae or pseudohyphae,[97,113,153,159,167,168,178–184] Using radiolabeled metabolic precursors, chemiluminescence, viable and nonviable *Candida*, and electron microscopy, Diamond *et al.*[97] were the first to demonstrate nonphagocytic damage to pseudohyphal forms of *C. albicans* by PMNL in the absence of serum. Initially, the PMNL spread over the hyphal surface to maximize membrane contact between the two forms. Fresh or heat-inactivated serum enhanced the process, but, in contrast to yeast forms, opsonization was not a requirement for the initiation of microbicidal processes by PMNL. The enhancement was related to anti-*Candida* antibody that was present in the sera. In subsequent reports, it was shown that damage to

hyphae was mediated by oxidative means, in particular by the MPO system.[167,168,178] Involvement of MPO was concluded from the following observations: 1) pseudohyphal damage was inhibited by sodium cyanide, sodium azide, catalase, superoxide dismutase and 1,4 diazobicyclo (2,2,2) octane, all agents known to act on PMNL oxidative mechanisms, 2) PMNL from 3 of 4 patients with CGD, a disease in which PMNL are produced that have defective microbicidal activity, and from 2 patients with hereditary MPO deficiency, were unable to damage hyphae, 3) pseudohyphal forms were severely damaged in a PMNL-free *in vitro* system containing MPO, H_2O_2 and iodide or chloride, and the H_2O_2 in the system could be replaced by an H_2O_2-generating system, and 4) methionine, tryptophan and alanine, compounds capable of inhibiting the effects of hypochlorous acid and chloramines, inhibited killing of pseudohyphae.

Induction of the oxidative burst and production of reactive oxygen microbicidal intermediates is a complex process involving events initiated at the PMNL membrane with subsequent involvement of secondary messengers and intracellular responses necessary to the effective function of the cell.[172,185] Delineating these aspects of the PMNL response to unopsonized and opsonized hyphae has been the principal focus of recent research on PMNL–*C. albicans* interactions by Diamond and his co-workers.[97,153,167,178–184] A number of these studies are summarized in Table I. Attachment of PMNL to opsonized or unopsonized hyphae, and spreading over them, appears to be independent of opsonins since both events occur at the same rate in the presence or absence of opsonins. Some of the events following attachment and spreading, however, occur at different rates and one of them, PMNL membrane depolarization, occurs only when opsonized hyphae are attacked.[180] Moreover, PMNL exposed to unopsonized hyphae manifest a delayed rise of cytosolic free Ca^{++},[180] and alterations in phospholipase C activation,[153] actin polymerization,[182] and initiation of superoxide anion release.[180] PMNL degranulation results in low levels of β-glucuronidase release and in lactoferrin release. Much of the released lactoferrin is found bound to hyphal surfaces and does not appear in the supernatant. Whether *in vivo* hyphae of *C. albicans* correspond more closely to the opsonized or unopsonized forms used in these *in vitro* experiments remains to be determined.

2.1.3d. Nonoxidative Anticandidal Mechanisms of PMNL. The ability of human leukocytes to kill some *Candida* spp. by nonoxidative mechanisms most likely reflects the presence of proteins and peptides with intrinsic antifungal activity. The major antimicrobial components of human PMNL are present in a subset of the cell's cytoplasmic granules known as the "primary" or "azurophil" granules. They include defensins, lysozyme, cathepsin G (a serine protease) and several proteins that are homologous to it.[186] Of these, only defensins and several of the serine protease congeners are active against *Candida* spp.

TABLE I
Selected Biochemical and Morphological Observations of Opsonized Zymosan and Opsonized or Unopsonized Hyphae of *C. albicans* during Interaction with Human PMNL

	Opsonized zymosan	Opsonized hyphae	Unopsonized hyphae
Attachment/ spreading[179]	Same kinetics	Same kinetics	Same kinetics
Intracellular Ca^{++}[179]	Peaked within 1 min; greatest concentration in periplasm	Peaked within 1 min; uniform distribution throughout hyphae	Peaked at 4 min; uniform distribution throughout hyphae
Membrane depolarization[180]	Ca^{++} increase first, then membrane depolarization, then respiratory burst	Ca^{++} increase first, then membrane depolarization, then respiratory burst	Ca^{++} increase delayed, no membrane depolarization, respiratory burst delayed
Role of GTP-binding proteins, sensitivity to pertussin-toxin[181]	Partial abrogation of normal Ca^{++}, membrane and respiratory burst responses	Partial abrogation of normal Ca^{++}, membrane and respiratory burst responses	Ca^{++} increase completely eliminated, respiratory burst intact
Inositol triphosphate generation[153]	Transient rise in cellular inositol phosphates	Transient rise in cellular inositol phosphates	Transient rise in cellular inositol phosphates
Actin polymerization[182]	Rapid, initially diffuse, then collarlike, then cylinderlike	Rapid, initially diffuse, then collarlike, then cylinderlike	Delayed, delayed appearance of collarlike, cylinderlike
Degranulation[182]	Low levels of β-glucuronidase detected	Low levels of β-glucuronidase detected	Low levels of β-glucuronidase detected
Specific granule marker[182]		Lactoferrin release into supernatant minimal, but it was found bound to surface	Lactoferrin release into supernatant minimal, but it was found bound to surface
Effects of chelation on respiratory burst[183]	Eliminated respiratory burst completely	Partially inhibited (60%) respiratory burst if both intracellular and extracellular Ca^{++} chelated, superoxide response inhibited	Partially inhibited (23%) respiratory burst; if both intracellular and extracellular Ca^{++} chelated, superoxide response unchanged

The human PMNL contains four defensins, which are designated HNP-1,2,3, and 4. Homologous peptides, called "lysosomal cationic proteins" were initially discovered approximately 25 years ago by Zeya and Spitznagel in the PMNL of rabbits and guinea pigs.[187] The human defensins are small (Mr < 4,000) peptides that contain 29 (HNP-2), 30 (HNP 1 and 3) or 33 (HNP-4) residues.[188] The known defensins and their primary amino acid sequences are presented in Table II. All defensins contain an invariant cysteine motif composed of six cysteine residues. These form three intramolecular disulfide bonds that fold the peptides into a complexly shaped cyclic structure, as reviewed elsewhere.[188,189] Although HNP-1, 2 and, 3 have identical primary sequences with the exception of their amino-terminal residues, only HNP-1 and HNP-2 kill *C. albicans* effectively *in vitro*.[190] HNP-1 and HNP-2 are remarkably abundant, and together they constitute approximately 4–5% of the total protein in human PMNL and 25–40% of the total protein in their azurophil granules.[191] HNP-4, which is substantially less abundant that HNP-1 and HNP-2, also exerts candidacidal activity *in vitro*.[192]

Rabbit[193] and rat[194,195] PMNL contain six and four defensins, respectively. Several of these defensins include *C. albicans* among their broad antimicrobial spectrum.[74,194,195] Although defensins have not yet been demonstrated in mouse PMNL, mRNA that codes for a typical defensin (cryptdin) is abundantly transcribed by cells that reside in the murine small intestine.[73] Based on *in situ* hybridization studies, the principal cells containing defensin message are epithelial (Paneth) cells located at the base of the intestinal crypts. Interstitial cells, presumably macrophages, in the small intestine's lamina propria also hybridized with the cryptdin probe.

The binding of defensins to *C. albicans* and the correlation of binding with candidacidal activity have been reported.[176] Several human, rabbit, and rat defensins kill *C. albicans* directly,[176,177] while some others were relatively ineffective alone but caused synergistic interactions when combined with submicromolar concentrations of the active peptides.[177] HNP-1 was rendered ineffective against *C. albicans* in an anaerobic environment and its candidacidal effects were also blocked by chemicals that blocked mitochondrial respiration by the yeast cell targets. These observations suggest that oxidative metabolism by *C. albicans* sensitizes it to human defensins. Although the reasons for the links between mitochondrial respiration and defensin-susceptibility remain to be established, recent studies showing that human and rabbit defensins produced voltage-sensitive membrane channels in artificial membrane bilayers may provide some clues.[196] If the *Candida* cell's own transmembrane potential provides the chemical energy used by defensins to create membrane damaging channels, the protective effects of mitochondrial inhibitors such as m-chlorophenylhydrazone (CCCP) or azide might result from a depolarization of the target cell membrane which prevents defensin channel formation.

TABLE II
Primary Amino Acid Sequences of Defensins Extracted from the Leukocytes of Various Species, Illustrating the Consensus of Seven Invariant Amino Acid Residues (Six Cysteines and a Glycine), as Shown by the Standard Single-Letter Code; Generally Conserved Residues are Indicated by Asterisks

Human	HNP-1	A C Y C R I P	A C I A G E R R Y G T C I Y Q G R L W A F C C
	HNP-2	C Y C R I P	A C I A G E R R Y G T C I Y Q G R L W A F C C
	HNP-3	D C Y C R I P	A C I A G E R R Y G T C I Y Q G R L W A F C C
	HNP-4	V C S C R L V	F C R R T E L R V G N C L I G G V S F T Y C C T R V
Guinea pig	GPNP	R R C I C T T R	T C R F P Y R R L G T C I F Q N R V Y T F C C
Rabbit	NP-1	V V C A C R R A	L C L P R E R R A G F C R I R G R I H P L C C R R
	NP-2	V V C A C R R A	L C L P L E R R A G F C R I R G R I H P L C C R R
	NP-3A	G I C A C R R R	F C P N S E R F S G Y C R V N G A R Y V R C C S R R
	NP-3B	G R C V C R K Q L	L C S Y R E R R I G D C K I R G V R F P F C C P R
	NP-4	V S C T C R R F	S C G F G E R A S G S C T V N G V R H T L C C R R
	NP-5	V F C T C R G F	L C G S G E R A S G S C T I N G V R H T L C C R R
Rat	RatNP-1	V T C Y C R R T	R C G F R E R L S G A C G Y R G R I Y R L C C R
	RatNP-2	V T C Y C R S T	R C G F R E R L S G A C G Y R G R I Y R L C C R R
	RatNP-3	C S C R T S	S C R F G E R L S G A C R L N G R I Y R L C C
	RatNP-4	A C Y C R I G	A C V S G E R L T G A C G L N G R I Y R L C C R
Consensus		C C *	C * * G C * C C

Abstracted from Lehrer, *et al.*[188]

In addition to its defensins, the azurophil granules of human PMNL contain several other polypeptides with antibacterial and antifungal activity.[186] The best studied of these are several isozymes of cathepsin G, a 25–28,000-dalton, highly cationic protein with chymotrypsin-like protease activity,[197,198] and a more recently described congener of this protein that has been named "azurocidin."[199–201] Although both azurocidin and cathepsin G are homologous to a large group of serine proteases, the fungicidal activity of CCCP was retained after inactivation of its protease activity,[174,197] and azurocidin itself is not enzymatically active. Recently, Shafer *et al.*[202] described the presence of several antimicrobial domains in cathepsin G and suggested that they contribute to the antimicrobial effects of the holoprotein. Extension of these studies to azurocidin and to *C. albicans* would be of considerable interest.

Other granulocyte constituents that may also contribute to host defense against fungi include lactoferrin and lysozyme (muramidase). Iron-free (apo)lactoferrin can, by tightly complexing iron necessary for optimal fungal growth, inhibit the growth of *C. albicans in vitro*.[63] Apolactoferrin, prepared from human colostrum, has been reported to kill *C. albicans in vitro*.[203] *Coccidioides immitis*[204] and *Cryptococcus neoformans*[205] are reportedly susceptible to killing by lysozyme, a hydrolase found in granulocytes and macrophages of most species, including man.[206] Killing of *C. albicans* by lysozyme is optimal in solutions of low ionic strength,[207] and may be facilitated by glucose.[208]

Finally, nonoxidative killing of *C. albicans* in skin may differ somewhat from that of other tissues. The importance of PMNL (and complement) in host defense against experimental cutaneous candidiasis was established by Ray and Wuepper,[209,210] but McNamara *et al.*[64] obtained evidence for the role of a small cytoplasmic protein in antifungal activity. They studied candidal lesions in the skin of mice. A clear zone existed between many of the more superficial pseudohyphae and the PMNL infiltrate in the deep dermis, suggesting that PMNL produced a soluble factor that inhibited the hyphae. Subsequently, they determined that lysates of PMNL contained a 30 kDa protein that was fungistatic for *C. albicans*.[64,65] The factor differs in several respects from previously described antimicrobial components of PMNL, e.g., it is found in the cytoplasm, not granules, and it appears extracellularly after the death or disruption of the PMNL.[64] Moreover, it appears to be fungistatic and not fungicidal,[65] although this has been contested.[66] It has been postulated that it may play a role in controlling the growth of *Candida* spp. in lesions prior to the time that intact PMNL can exert candidacidal effects.

2.1.3e. Alternation of PMNL Function by C. albicans. Despite the fact that normal PMNL are relatively effective candidacidal agents, the fungus may have considerable potential to subvert PMNL activity. Louria and Brayton[211] demonstrated that phagocytized *C. albicans* yeasts that were not killed by PMNL subsequently germinated, penetrated the cell membrane and actually

grew out of the leukocyte. Diamond *et al.*[97,212] demonstrated that *C. albicans* hyphae released small peptides, 2500–3000 kDa, which inhibit the adherence process for not only *C. albicans*, but also *Rhizopus* spp., *Aspergillus* spp., and some bacteria. Smail *et al.*[213,214] reported that a crude hyphal inhibitory product (CHIP) produced by *C. albicans* inhibited superoxide anion production and release of azurophil and specific granule components by FMLP-stimulated PMNL. It is not clear whether or not CHIP and the small peptide(s) described earlier are the same molecules. Hilger and Danley[215] and Danley *et al.*[216] noted that PMNL released decreasing amounts of H_2O_2 when incubated with viable yeasts, whereas increasing amounts were released in response to dead organisms. Although inconclusive, the data suggest the possibility that *C. albicans* may somehow modulate PMNL activity. Finally, the intriguing observations of complement receptors on the surface of *C. albicans*[217–221] have led to various speculations regarding their role in pathogenesis. Receptor expression was reportedly increased when the organisms were grown at glucose levels that prevail in hyperglycemic subjects.[222,223] A relationship between complement receptors and virulence has been established, but their role in pathogenicity has not been determined definitively.

Finally, PMNL appear to promote phenotypic switching in *C. albicans*,[170] an event which may allow the fungus to escape destruction by the PMNL. Phenotypic switching, a phenomenon first described by Slutsky *et al.*,[224] involves inheritable spontaneous changes in cellular and colonial morphology which occur at a relatively high frequency. The relationship between switching and pathogenicity is problematic at this stage, but, since changes in coagglutination and adherence to epithelial cells occur during switching, it has been suggested that switching may play a role in disease manifestations.[225]

2.1.4. Eosinophils

Human eosinophils ingest and kill *C. albicans in vitro* at a rate similar to neutrophils and their phagocytic activity also depends on immunoglobulin and complement components.[162,226,227] Eosinophils from subjects with CGD have an impaired ability to kill *C. albicans*.[162]

2.1.5. Monocytes and Macrophages

2.1.5a. Interactions between Human Mononuclear Cells and C. albicans. In contrast to the strong evidence that identifies PMNL as key cells in innate defenses against systemic *C. albicans* infections, the role of monocyte (MN) and macrophage (MØ) in resistance is not as well defined. Data on interactions of human MØ with *Candida* spp. are relatively limited. The candidacidal activity of MN in patients with various disorders has been studied by relatively few investigators, which is unfortunate given the ease whereby such

studies can be performed.[96,228] MN from patients with lepromatous leprosy[229] and AIDS[230] were reported to be markedly deficient in their ability to kill *C. pseudotropicalis*. These interesting findings warrant verification and further exploration. Normal human MN were reported to kill 63.4 ± 10.2% of ingested *C. albicans* in 2.5h when they were tested in the presence of normal PMNL.[96] Under these conditions, normal human MN also killed 46.5 ± 3.7% of ingested *C. parapsilosis* and 65.7 ± 2.9% of ingested *C. pseudotropicalis* in 3h. When normal MN were tested under PMNL-free conditions, they were less effective against *C. albicans* but still killed them about as effectively as purified PMNL. These data indicate that human blood MN have the capacity to contribute significantly to host defense against *C. albicans*.

Human alveolar MØ utilize energy provided by both their oxidative and glycolytic metabolism to ingest *C. albicans*.[231] Although they were able to kill ingested *Listeria monocytogenes*, they failed to kill yeast cells of *C. albicans*.[231,232] Human alveolar MØ killed 18% of *C. pseudotropicalis* in 90 min, with equivalent performances given by cells derived from smokers and nonsmokers.[233] Alveolar MØ from human bone marrow transplant recipients[234] and from subjects with pulmonary alveolar proteinosis[235] were reported to have a decreased ability to kill ingested *C. pseudotropicalis*. MØ from human milk could ingest *C. albicans* in the presence of milk, but their candidacidal activity was not determined.[236] Studies of tissues from patients with systemic candidiasis have shown damaged-appearing *Candida* within fixed MØ.[237] Although such findings are consistent with the intrinsic fungicidal competence of MØ, they could also result from the uptake of *Candida* cells that had been killed by other host factors. MØ that were derived by cultivating human blood MN *in vitro* retained their ability to take up and digest heat-killed *C. albicans*[238] but lost their ability to kill viable *C. albicans* yeast cells.[239,240]

Prostaglandin E_1 and theophylline, substances that modulate intracellular cAMP levels, inhibited the candidacidal activity (*C. albicans*) of human PMNL[241] and MN.[96] *In vitro*, phenylbutazone was a potent inhibitor of human PMNL[94] and monocyte candidacidal[96] and digestive activity.[242] As hydrocortisone lacked such activity in granulocytes,[94,110] the ability of such corticosteroids to inhibit chemotactic entry of leukocytes into *Candida*-infected tissues may underlie its *in vivo* impairment of anti-*Candida* defenses.[243,244] Corticosteroids were reported to inhibit candidacidal activity by human MN *in vitro* in some experiments[245] but not in others.[96] *Escherichia coli* endotoxin reportedly reduced ingestion and degradation of ^{125}I-labeled *C. albicans* by human MN.[246]

The candidacidal mechanisms of human MN are partially understood, based largely on studies with cells that were obtained from patients with hereditary MPO-deficiency or CGD.[96] The ability of MPO-deficient MN to kill *C. albicans* was greatly impaired, but such cells killed both *C. parapsilosis* and *C. pseudotropicalis* more effectively than did normal MN. In sharp contrast, MN that were unable to generate O_2-(superoxide anion) and H_2O_2

(obtained from patients with CGD) failed to kill any of these three *Candida* species. Human MN were also shown to contain components that killed *C. parapsilosis in vitro*,[96] but these have not been identified precisely and their participation in MN-mediated anticandidal mechanisms remains to be demonstrated. In summary, the available data indicate that a) normal human MN can kill *C. albicans* at least as effectively as PMNL, b) their candidacidal mechanisms are linked to the production of oxidants, c) the candidacidal mechanisms of human MN are evidently mediated by both MPO-dependent and MPO-independent pathways, and d) human MN contain potentially candidacidal components. Based on the studies with MPO-deficient MN, MPO-dependent oxidative mechanisms appear to be most important with respect to *C. albicans*, while MPO-independent oxidative mechanisms suffice to enable MN to kill *C. parapsilosis* and *C. pseudotropicalis*. It should be noted that the nonoxidative candidacidal effectors discussed previously with respect to human PMNL, including defensins[247] and cathepsin G[248] are not present, or are present only in small amounts in blood MN.[248]

2.1.5b. In Vivo Studies of Mononuclear Cell–Candida spp. Interactions in Murine Models. Several investigators have used mice treated with macrophage suppressants such as carrageenan[249] or silica[250,251] to abrogate reticuloendothelial activity, or mice with genetically defective macrophage activity,[252] to determine the role of MØ in resistance to systemic candidiasis. Unfortunately, most suppressive treatments induce multiple abnormalities and responses in many genetically altered animals are not limited to a single defect, so that interpretation becomes problematic. For example, carrageenan may increase resistance against systemic candidiasis[249] by virtue of the fact that it stimulates granulocytosis. Athymic nude mice, animals that reportedly have increased numbers of activated MØ,[253] are more resistant to an initial challenge with *C. albicans*,[254] and, further, Cutler and Poor,[84] using a unique chamber implanted subcutaneously into mice,[255] determined that MØ from nude mice were candidacidal, whereas those from normal mice were only candidastatic. The enhanced resistance was only temporary, and death ultimately occurred from infection. Lee and Balish,[250] however, comparing nude mice and their normal littermates treated with silica found no differences between nude and normal mice, i.e., the nude mice were neither more resistant nor more susceptible. Finally, Bistoni *et al.*[256] using splenocytes from mice immunized with an avirulent *C. albicans*, transferred protection to recipients challenged with a virulent *C. albicans* with an adherent spleen cell population.

Other experimental approaches have also been adopted in an attempt to define the contributions of MN and MØ in resistance to candidal infections. In many investigations, cyclophosphamide (CY)[82,257–261] or the related compound, nitrogen mustard[262,263] were used as the suppressive agents. In all cases cited, CY or nitrogen mustard promoted enhanced mortality or tissue destruction when animals were challenged with *C. albicans*. In one of the

studies,[258] the investigators were able to demonstrate striking similarities between *in vivo* susceptibility and depressed *in vitro* measures of immunologic function, including assays for PMNL function, natural killer (NK) cell function, and alloreactivity. Moreover, although CY-treated mice were highly susceptible to *C. albicans* challenge a few days following CY administration, 2–3 weeks following CY administration the animals were actually more resistant than normal to challenge with the fungus. This resistance could be explained on the basis of the rebound effect, i.e., as CY is cleared from the system and cell division proceeds, PMNL are produced at a greater-than-normal rate so that their levels exceed normal values for that interval. Since CY and nitrogen mustard are alkylating agents which nonspecifically damage dividing cells, data based on their use must be interpreted with care. PMNL would certainly be the most severely affected, but MN and other lymphoid cells are affected as well.

A number of other investigations have focused on the ability of macrophage activators such as LPS and *Bordetella pertussis*,[264] *Listeria monocytogenes*,[265] Bacille Calmette Guerin (BCG),[251,266,267] *Corynebacterium parvum*,[266,268] or muramyl dipeptide[269–271] when administered *in vivo* to increase resistance against an intravenous challenge with a potentially lethal dose of *C. albicans*. On balance, in most studies the stimulator induced resistance to *C. albicans* that was demonstrable immediately after the candidal challenge, but once a chronic infection was initiated, the disease progressed and many additional deaths occurred. In several cases,[265,270,271] disease progression could be halted if the stimulator was administered repeatedly throughout the course of the experiment.

Some recent observations suggest that immature macrophage precursors may protect mice during experimental *C. albicans* infections. Approximately two weeks after receiving CY, mice developed a highly candidacidal population of spleen cells which were nonadherent to nylon wool and appeared to be macrophage precursors.[258,272,273] Moreover, a similar population was found in the liver of CY-treated animals[274] and in bone marrow cultures established with the L-929 cell line.[275] Using these data and observations concerning cell-surface antigens and the effects of colony-stimulating factor, these investigators concluded that a highly candidacidal but immature precursor macrophage population occurred in the spleen, liver and bone marrow. When they were allowed to differentiate into mature MØ, the cells lost their candidacidal properties.[275,276] The investigators speculated that if colony-stimulating factors were generated *in vivo* during infection, the cells could be maintained in a perpetual, immature (and candidacidal) state that could contribute significantly to anti-*Candida* activity.

Some data concerning *in vivo* candidacidal activity of MØ is available. By 15 min after injection into the tail vein, *C. albicans* could be found within murine hepatic MØ, and after 48h the ingested yeasts displayed altered staining characteristics, and granulomas, possibly glucan-induced, persisted

for weeks.[277] Normal rat livers cleared *C. albicans* from perfusates, but their candidacidal activity was negligible. Livers from animals that received prior vaccination with *Corynebacterium parvum* killed approximately 40% of the cleared *C. albicans*. This fungicidal activity was reversed by silica, phenylbutazone, and iodoacetate.[268] Phenylbutazone, but not iodoacetate, was reported to inhibit killing of *C. albicans* by human blood MN.[96] In contrast, iodoacetate, but not phenylbutazone, blocked killing of *C. albicans* by rabbit lung MØ.[278] Such discrepancies emphasize the heterogeneity of mononuclear phagocyte fungicidal mechanisms according to host species and/or tissue location.

2.1.5c. Pulmonary Resistance Mediated by Mononuclear Cells in Rabbit and Murine Models. Because *C. albicans* normally resides in the mouth, aspiration of this organism into the lungs probably occurs with some frequency. Nevertheless, pneumonias caused by *C. albicans* are extremely rare, suggesting that the lungs and respiratory tract are well defended against this organism. Blastospores that are injected into peripheral veins of rabbits, which are highly resistant to infection by *C. albicans*, are cleared principally in the lung, while those injected into mesenteric veins are chiefly removed by the liver.[77] Such hepatic clearance is probably responsible for the initiation of hepatic candidiasis in neutropenic subjects with cancer or leukemia.[279–281]

Experimental models have been developed in rabbits and mice to investigate the potential for the alveolar MØ to deal with *C. albicans*. Rabbits that receive up to 10^8 blastospores intratracheally develop a bronchopneumonia that clears within 5 days without leaving residual damage.[282] Although the mechanisms responsible for pulmonary resistance are not certain, rabbit PMNL contain large concentrations of defensins,[174] several of which are potently candidacidal.[74] Rabbit alveolar MØ can also exert candidastatic and candidacidal activity[283] and possess lysosomal components that are inhibitory to *C. albicans*.[284] Peritoneal and alveolar MØ recovered from rabbits that had been stimulated *in vivo* with complete Freund adjuvant (CFA) showed enhanced candidacidal activity relative to their control, unstimulated counterparts.[278] Patterson-Delafield *et al.*[285] identified two peptides, initially designated as MCP-1 and MCP-2 (macrophage cationic peptide), in rabbit alveolar MØ. The peptides were potently candidacidal *in vitro* against *C. albicans* as well as against a variety of gram-positive and gram-negative bacteria. MCP-1 and MCP-2 were later sequenced[286] and shown to be structurally identical to two of the six defensins (NP-1 and NP-2) that are expressed in rabbit PMNL.[193] Neither MCP-1 nor MCP-2 was present in rabbit blood MN or peritoneal MØ, and the peptides were present in greatly reduced amounts in the pulmonary MØ of neonatal rabbits,[287] which have a reduced ability to kill ingested *C. albicans*.[288] Cell-free fluid recovered from rabbit lungs by bronchoalveolar lavage contains a 10 kDa agglutinin for *C. albicans*[289] whose significance for resistance to pulmonary candidiasis is uncertain.

Other investigators have examined candidacidal activity in rabbit MØ as

well. Arai *et al.*[290] compared alveolar MØ obtained from normal and *C. albicans*-immunized rabbits. They reported that immune MØ in immune serum displayed the most effective ingestive powers, and that immune and control MØ displayed equivalent candidacidal activity. Another study[278] compared resident MØ with MØ elicited by prior *in vivo* injection of CFA. Resident (unelicited) alveolar MØ destroyed approximately 28% of ingested *C. albicans* after 4h, whereas resident peritoneal MØ killed 15% after 4h. Peritoneal MØ from animals pretreated with CFA manifested enhanced candidacidal activity (28% in 4h), and CFA-elicited alveolar MØ killed 32% of ingested *C. albicans* after 4h. In a limited study[291] of antecedent BCG stimulation, BCG-elicited rabbit peritoneal and alveolar MØ showed candidacidal activity for *C. albicans* that was similar to that of their unstimulated counterparts. In the study in which intracellular vital staining with methylene blue was employed, 42–45% of ingested organisms were killed in 1h. Using an assay based on [^{3}H] leucine incorporation, there was 71–93% inhibition of macromolecular synthesis by *C. albicans* that had been ingested by alveolar MØ. The latter findings were consistent with fungistatic and/or fungicidal activity.[283]

In mice, Evans[292] reported that *C. albicans* blastospores and hyphae injected intravenously were killed in lungs more effectively than any other organ. Hyphae were eliminated from lung sections by 24h without detectable inflammatory response. Swiss-Webster mice uniformly survived the intratracheal administration of 3×10^6 blastospores, a dosage that killed >85% of the mice if injected intravenously.[293] After a 6h delay, the instilled organisms were cleared rapidly (>90% in 24h), and this rapid pulmonary clearance was inhibited in steroid-treated mice. Intratracheal immunization with *C. albicans* two weeks prior to intratracheal challenge was reported to enhance pulmonary clearance,[294,295] which was thought to be mediated largely by PMNL recruited into the lungs during the infection.

A potentially important contribution to understanding pulmonary resistance to *Candida* infections was made by Nugent and Fick,[296] who reported that cell-free bronchoalveolar lavage fluid from Swiss-Webster mice contained a protein that killed *C. albicans*, *C. tropicalis*, and *C. glabrata*. The protein had an apparent molecular weight of 29 kDa and was not active against *C. parapsilosis* and *C. krusei*. The precise identity of the protein, its cell of origin, and its relationship to similarly sized candidacidal effectors, such as cathepsin G and azurocidin, that have been described in human PMNL remain to be determined.

Several factors contribute to the overall ability of pulmonary mononuclear cells to deal with *C. albicans*. For example, antifungal activity of alveolar MØ may be impaired by antecedent viral infections. Normal mouse alveolar MØ killed 54.5% of *C. krusei* in the presence of nonimmune serum and complement factors, but those from mice with parainfluenza-1 virus infections showed significantly diminished candidacidal ability, despite intact

powers of ingestion.[297] Moreover, alveolar MØ obtained from neonatal rhesus monkeys were markedly impaired in their ability to kill *C. albicans* compared to MØ obtained from adult monkeys.[298] Impaired candidacidal activity has also been reported in alveolar MØ obtained from neonatal rabbits[288] and may be a reflection of the low levels of defensins (MCP-1 and MCP-2) and defensin mRNA in these cells.[287]

Brummer and Stevens[299] demonstrated that although resident murine pulmonary MØ lacked the ability to kill ingested *C. albicans* blastospores, MØ that had been cultured overnight with lymph node cells and concanavalin A (con A) or con A-splenocyte supernatants killed 24–43% of the organisms. Recombinant interferon-gamma (IFN-γ) failed to activate candidacidal activity in these MN, although it markedly enhanced the candidacidal performance of peritoneal MØ obtained from the same animals.

2.1.5d. Interaction of Murine Peritoneal Mononuclear Cells with C. albicans. Murine peritoneal MØ have been used in the majority of studies dealing with the activities of animal MØ against *C. albicans*. In an early study with resident mouse peritoneal MØ that had been grown in tissue culture medium for 24h, Stanley and Hurley[300] found that the cells rapidly ingested six species of *Candida* in the presence of 10–20% homologous serum and that within 2h, intracellular replication by germ tubes (*C. albicans*) or budding (other *Candida* spp.) had occurred. By 24h, MØ cultured with *C. albicans* or *C. tropicalis* had been destroyed, while those incubated with *C. parapsilosis*, *C. pseudotropicalis*, *C. krusei*, and *C. guilliermondii* remained viable for an additional 24–48h. The authors concluded that MØ were unlikely to contribute substantially toward host defenses against disseminated candidal infections. Ozato and Uesake[301] used [3H] leucine or [3H] uridine uptake with radioautography to study the ability of caseinate-elicited mouse peritoneal MØ to inhibit *C. albicans*. By 2h, ingested organisms displayed no isotope uptake, despite significant incorporation by extracellular fungi. Intracellular growth of *C. albicans* commenced by 3h and resulted in rapid destruction of the MØ.

In a murine model[84] wherein diffusion chambers with different pore sizes (0.45 and 3 microns) were implanted intraperitoneally, killing of *C. albicans* required phagocytic cells. Thioglycollate-induced peritoneal MØ from BALB/c mice restricted growth of *C. albicans* but did not kill them. MØ from nude mice reduced the number of *C. albicans* by 80–90% in 24h when added at high (> 40:1) macrophage:yeast cell ratios.

An *in vitro* colony-counting assay was used to compare resident peritoneal MØ with cells obtained from LPS- or BCG-treated mice.[302] Resident MØ killed 10% of *C. albicans* in 3h, compared with 27% for LPS-elicited cells and 23% for BCG-elicited cells. In contrast, *C. parapsilosis* was killed much more efficiently, as follows: resident MØ, 72%; LPS-elicited cells, 84%; and BCG-elicited cells, 81%. Muramyl dipeptide, a synthetic adjuvant, increased fungicidal activity of overnight-cultured mouse peritoneal MØ against *C.*

albicans in vivo[269] and *C. parapsilosis in vitro*.[303] Maiti *et al.*[304] also showed that peritoneal MØ obtained from BCG-vaccinated Swiss mice more effectively resisted the intracellular germination of ingested *C. albicans* than did MØ obtained from control mice and that exposure of the MØ to crude lymphokine mixtures enhanced their candidastatic properties. The ability of most ingested *C. albicans* blastospores to survive after their ingestion by resident peritoneal mouse MØ cannot be ascribed to the failure of phagosome-lysosome fusion.[305]

Brummer *et al.*[306] demonstrated that murine MØ pulsed overnight with recombinant murine IFN-γ, but not with LPS, exhibited significantly enhanced fungicidal activity against *C. albicans*, relative to control MØ that had been incubated overnight with medium (44% versus 0% killing). Enhanced candidacidal activity was also observed when the MØ were treated with supernatants from con A-treated spleen cells or with con A itself. This effect was abrogated by including antibody to IFN-γ in the incubation mixtures. Kolotila *et al.*[307] demonstrated that *in vivo* (intraperitoneal) administration of con A to mice enhanced the candidacidal activity of their peritoneal MØ and described sex and strain-specific factors that influenced macrophage performance.

2.1.5e. Summary. In summary, most of the *in vitro* data suggest that activated MØ are more candidacidal than resident cells.[299,302,304,307–310] Unheated serum, with or without *Candida*-specific antibody, promoted phagocytosis,[77,127,268,290,309] but did not appear to increase killing capacity.[127,290] Despite the fact that activated cells appear to be more candidacidal than resident cells, the level of killing observed is modest, even by activated cells, and suggests that many *Candida* survive their initial encounter with these cells. The implications of these seemingly "low" levels of leukocyte-mediated killing on clearing foci of *C. albicans* from infected tissues is considered in Section 2.1.7. Although much remains to be learned about the mechanisms by which MØ kill *Candida* spp., they are likely to include oxygen-dependent[96,240,302,311] and oxygen-independent pathways[96,278,284,285,311–313] whose primacy may vary according to the cell's site and species of origin[261] and their state of activation. Genetic or sex differences such as those observed among mouse strains may also contribute.[272,307] Consequently, the often divergent data summarized in these studies may have multiple causes in addition to those mentioned previously, which include use of different assay conditions, effector: target ratios, incubation periods, viability tests, etc.

2.1.6. NK Cells

Evidence describing potential interactions of NK cells with other host cells and *C. albicans* is reviewed elsewhere in this volume and will not be discussed here.

2.1.7. Overview of Cellular Defenses

Elimination of infections caused by *C. albicans* will occur over time if the organisms are killed more rapidly than they reproduce. The experimental data reviewed above demonstrates that phagocytic cells, specifically PMNL, MN, and MØ, have the ability to kill a fraction of the *C. albicans* that they encounter. This fraction may vary from 10 to 50% or more, depending on the type of phagocyte and the experimental conditions employed. A feature (some would say defect) of the *in vitro* assays used to derive these data is that they measure only the initial results of the interaction between the fungus and phagocyte(s). *In vivo*, fungi that survive the initial round of this struggle undoubtedly continue to encounter additional phagocytes until the issue is resolved. Although a net reduction in the number of fungi by 15% or 20% per phagocytic encounter may not seem a highly promising foundation for host defense, the theoretical calculations illustrated in Fig. 1 suggest otherwise. In composing this figure, it was assumed that the average battle cycle between an individual *C. albicans* and a phagocyte lasted for 6h, and that four such interactions occurred each day for each surviving organism. The curves indicate overall *C. albicans* survival, and are predicated on net reductions of 5%, 10%, or 15% of the organisms/cycle. Note that as little as 15% net killing/cycle would effectively clear the infection within a week. Although Figure 4.1 is theoretical, it suggests how relatively small degrees of enhanced candidacidal activity could reap large "dividends" in overall host resistance and why a system that combines candidastatic and modestly effective candidacidal components normally has such high efficacy.

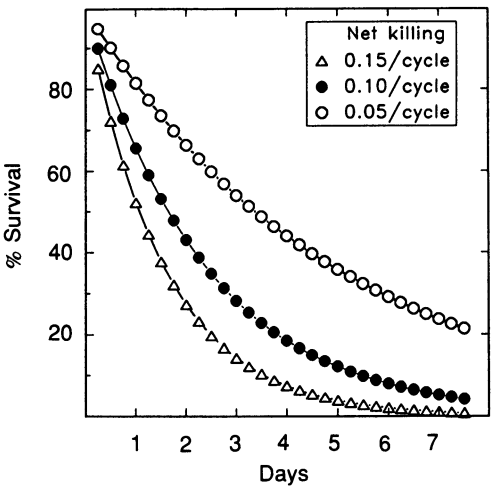

FIGURE 1. This figure is a hypothetical curve for the clearance kinetics of a hypothetical population of *C. albicans* subjected to 4 daily cycles of combat with host phagocytes. To construct these curves, it was assumed that each cycle of *Candida*-phagocyte interaction lasted for 6 h (4 cycles/day) and resulted in the net killing of 5% (○), 10% (●), or 15% (△) of the surviving organisms/cycle. The curves show that "nibbling away" at organisms in this manner can eliminate them and also that relatively small increases in net killing efficiency would greatly accelerate clearance.

2.2. Acquired Immune Responses

2.2.1. Protective Immunity

Despite the fact that a number of investigators have been attempting for many years to dissect specific immune responses to *Candida* spp. to determine their relationship to protection, protective immunity in candidiasis remains an enigma. Confusion over this issue relates to several problems. First, many researchers have investigated innate immunity and attempted to extrapolate the data to acquired immunity. Secondly, several investigations have been carried out wherein mice have been immunized with nonviable blastoconidia[294,314–316] or subcellular preparations[314,315,317,318] of *C. albicans*, which likely have no relevance to the development of protective immunity in the intact individual. In general, greater protective responses have been achieved when animals were immunized with viable preparations of virulent[319,320] or avirulent[256] organisms. Thirdly, it has been recognized only recently that protective immunity against mucocutaneous candidiasis appears to differ fundamentally from that against systemic disease. Fourthly, since defense against primary fungal pathogens such as *Histoplasma capsulatum* and *Coccidioides immitis*, organisms that produce systemic disease, was determined early on to be primarily cell-mediated, there was a built-in bias that immunity in candidiasis should also be cell mediated. The accumulated evidence, however, as summarized below, favors the interpretation that protective immunity in candidiasis is a complex response involving both cellular and humoral mechanisms in conjunction with innate immunity, especially the PMNL. Cellular immunity appears to be more critical in defense against mucocutaneous disease, as summarized in Chapter 5, than against systemic disease.

Evidence supporting a role for antibody in protection against systemic candidiasis has been provided at several levels, namely, immunization designed to elicit antibody prior to challenge,[315,321] reports of transfer of protective responses to naive recipients with serum from immunized animals,[321–324] treatments intended to abrogate the ability of animals to produce antibody prior to immunization and challenge,[325,326] and the correlation of clinical conditions in humans with an antibody response to a specific antigen detected by the Western blotting technique.[327] None of the data have provided definitive proof of the assertion that antibody is protective, however.

For example, in the studies by Mourad and Friedman,[322] antibody was administered repeatedly, and when antibody administration was terminated, mice began dying at a rate similar to that of the controls. In the studies by Pearsall *et al.*,[324] assessment of protection involved the measurement of the thickness of the thigh, comparing treated and control animals; no cultural assays were performed. Since Moser and Domer[325] have shown that two lesions of equal volume can contain very different numbers of organisms, it is not clear whether or not lesion size is an accurate reflection of protection.

Further, Scheld *et al.*[321] were not actually investigating systemic candidiasis, they were working with a model of experimental endocarditis. In such a model, where attachment to the target tissue would be critical to the establishment of disease, one can readily envision the prevention of disease in the presence of antibody to surface antigens. The CY model employed by Moser and Domer,[325] wherein the CY was administered in such a way as to abrogate antibody production but leave cellular immunity intact, provides circumstantial data supporting the role of antibody, as does the study of Matthews *et al.*[327]

Perhaps the model that held the most promise initially in experimental efforts to determine the role of antibody in protection was that involving anti-μ treatment of mice.[326] Here again, however, the data did not provide a definitive answer to the question. Female mice treated with anti-μ were partially protected after immunization but male mice were not. Female mice are innately more resistant to *C. albicans* than males,[328] and the data support the hypothesis that the innate defenses, antibody and cellular immunity are all necessary to complete protection against systemic candidiasis. Several studies involving immunosuppressed animals performed in a single laboratory are summarized in Table III to illustrate the fact that both T cells and antibody appear to be important in successful defense against *C. albicans*, but that the specific role of each has not yet been identified.

Although the studies described above would suggest a role for antibody in protection, there are examples in the literature of studies that illustrate just the opposite.[329–331] Moreover, attempts to illustrate the involvement of antibody in killing *in vitro* have yielded conflicting data. Morelli and Rosenberg[123] and Morrison and Cutler[127] found no evidence for enhanced intracellular killing in the presence of *C. albicans*-specific antibody, whereas Maiti *et al.*[304] and Pereira and Hosking[132] found that immune serum enhanced killing.

Included in Table III are data from a study involving mice thymectomized and irradiated at an early age, then reconstituted with bone marrow.[332] Such mice were neither innately more susceptible nor more resistant to *C. albicans*, but neither were they protected against systemic challenge following immunization. It is clear from this study and others[330,333–336] that the T lymphocyte is involved in acquired immunity, but its precise role remains to be elucidated. In the two most recent publications,[335,336] mice immunized with an avirulent mutant and then challenged with a virulent mutant were given anti-L3T4 or anti-Lyt-2.2 after immunization and immediately prior to reinfection for assessment of a protective response. As assessed by quantitative culture of kidneys, the antibody-treated mice were significantly more susceptible to the virulent *C. albicans* than were mice treated with medium alone. Taking all the data into consideration, however, the effect of the anti-T cell reagents or T-cell products seemed to be one involving the kinetics of the response not the final outcome, in that even the animals treated with anti-T cell reagents eventually cleared *C. albicans* from their tissues.

TABLE III
Correlation of Protective Immune Response to *C. albicans* with Demonstrable Antibody to Cytoplasmic Substances or DH

		14–28 days post-IV				
	Sex	CIE +/TOTAL	ELISA	DH	Protection	Reference
Normal						320
Imm	M	36/39	2.73 (0.17)	Yes	Yes	
Uunimm	M		1.70 (0.00)	No	No	
Thymectomized (TX)						332
TX Imm	M	2/30		No	No	
sham Imm	M	13/30		Yes	Yes	
normal Imm	M	41/90		Yes	Yes	
Cyclophosphamide (CY)-treated						325
CY Imm	M	3/36		Yes	No	
normal Imm	M	35/42		Yes	Yes	
Anti-mu(μ)-treated						326
μ Imm	M	0/5	0.30 (0.30)	Yes	No	
μ Imm	F	0/6	0.17 (0.17)	Yes	Partial	
μ Unimm	M	0/6	0	No	No	
μ Unimm	F	0/10	0.17 (0.17)	No	No	
Mock-Imm	M	7/7	2.88 (0.09)	Yes	Yes	
Mock-Imm	F	7/7	2.68 (0.20)	Yes	Yes	
Mock-Unimm	M	0/10	0.30 (0.30)	No	No	
Mock-Unimm	F	0/11	0	No	No	

In conjunction with studies attempting to detail the role of lymphoid cells in resistance to infection or reinfection, some investigators have considered lymphokine production,[335,337] lymphokine administration,[338] or transfer of immunity with crude mixtures such as dialyzable leukocyte extract.[339] Neutralization of endogenous IFN-γ slowed the rate of clearance of *C. albicans* in immunized animals,[336] but the administration of exogenous IFNγ to unimmunized mice challenged with *C. albicans* was somewhat suppressive, resulting in increased growth of the organism in selected tissues.[338] Administration of IFNγ to immunized mice had no effect, however. The administration of human dialyzable leukocyte extract to mice, effective in only a few instances, appeared to result from nonspecific effects of the extracts.[339] Studies involving the administration of various cytokines to animals in attempts to modify their responses to *C. albicans* are in their infancy, and data generated by such studies are difficult to interpret. It is clear that multiple cytokines are produced during immune responses and several have been shown to have synergistic effects *in vitro*. Only after considerably more is known of *in vivo* cytokine production, can the effects of exogenously applied cytokines be evaluated effectively.

Since the delayed hypersensitivity (DH) response is a measure of one aspect of cellular immunity, several investigators have based their conclusions on the role of T lymphocytes in protection on correlations between DH levels and protection. Kagaya *et al.*,[340] Sinha *et al.*,[341] Cenci *et al.*,[335] and Ashman[342] have all suggested that since DH levels parallel resistance, cell-mediated immunity is clearly involved in protection. Hurtrel *et al.*,[343] however, noted an absence of correlation between DH and protective immunity in the murine system. Unfortunately, the reagents that are used to abrogate T-cell activity do not appear to be highly selective for subsets of T cells, and until it has been clearly established that the T cells involved in DH are the same as those involved in protection, conclusions regarding the relationship between DH and protection are premature.

As indicated previously, a number of investigators have attempted to determine the role of lymphoid cells in protection by assessing response to a primary exposure to *C. albicans* in immunologically modified animals.[72,134,254,333,341,344–351] In many instances, the defects observed, both in genetically deficient animals and in artificially induced immunologically modified animals, are multiple and interpretation of the data is complicated. For example, Cutler[254] and Rogers *et al.*[346] both reported enhanced resistance to primary challenge in congenitally athymic mice, thus suggesting that T cells were not critical to defense against *C. albicans*. On closer inspection, however, the resistance observed was evaluated after challenge with either low or high levels of *C. albicans*, and the data could be explained on the basis of increased numbers of activated macrophages observed in athymic mice, not necessarily T lymphocytes. Interestingly, Mahanty *et al.*,[349] working with the severe combined immunodeficient mouse model, noted little difference between normal and immunodeficient mice and concluded that neither T nor B cells were necessary for innate resistance to a primary challenge with *C. albicans*. Recently, Cantorna and Balish[72] found that only congenitally deficient mice that were defective in both T-cell activity and phagocytic activity were susceptible to the development of mucosal candidiasis that spread systemically.

Finally, several groups of researchers[138,347,350,352–357] have taken a genetic approach to investigations of innate murine susceptibility to *C. albicans*. Not surprisingly, resistant and susceptible mouse strains have been identified and genes affecting the response have been mapped both within[347,356] and outside[354] the major histocompatibility complex. Despite the fact that there are clear differences in innate susceptibility, protective responses following immunization could be demonstrated in all 6 mouse strains investigated by Hector *et al.*[138] An interesting new development in studies of this type is the observation that one strain of genetically susceptible mouse, the CBA/H strain, develops not only weaker pathologic and immune responses to *C. albicans*, but their cells can also be stimulated *in vitro* to develop autoreactive cells, i.e., cells that, when then administered *in vivo*, are cytotoxic to apparently unaltered syngeneic host cells.[357] They suggest that "molecular mim-

icry" exists between *C. albicans* and host tissue antigens, which may contribute to pathogenesis. Since in-depth genetic studies of resistance to *Candida* spp. are a relatively recent innovation, it is not yet clear how they apply specifically to innate versus acquired defenses.

2.2.2. *Immunoregulation*

It is clear from clinical data[358] that some patients with candidiasis have depressed immune function, especially cellular immunity, demonstrable *in vivo* as skin testing for DH, or *in vitro* by lymphocyte stimulation assays. The most profound immunosuppressive effects are usually *Candida* specific, but some patients show generalized or partial anergy when tested with noncandidal antigens. The relationship between suppressed activity and disease is not clear, in that cause and effect relationships have not been defined. Most patients in whom suppressed responses have been described have had mucocutaneous, not systemic, disease, either in the form of chronic mucocutaneous candidiasis[358] or recurrent vaginitis.[359] The fact that the clinical observations are skewed toward mucocutaneous disease may be misleading with respect to the candidal immunoregulatory phenomena in general, however, in that patients with systemic candidiasis, in contrast to those with mucocutaneous disease, are often not evaluated immunologically.

The working hypothesis for researchers investigating immunoregulatory phenomena is that one or more components of *C. albicans* seeps into the circulation and initiates a series of events that culminate in reduced immunologic activity. In fact, serum factors, presumably responsible for some or all of the immunosuppression observed, have been found in many patients. The nature of the putative inhibitors has not been defined clearly in each case, but there is evidence[358] that a polysaccharide, in some instances mannan specifically, may have been the cause of the immunosuppression in a subset of the patients. Contrary to the polysaccharide theory, however, in the one instance where patients with disseminated disease were investigated,[135] the immunosuppressive agent in the sera of seven patients appeared to be immunoglobulin. The inhibitor in the sera of those patients was demonstrated in a PMNL candidacidal assay. When patient sera were included in the assay, candidacidal activity was reduced from a mean of approximately 18% to $< 5\%$ in most cases. Moreover absorption of patient sera with heat-killed *C. albicans* abrogated suppressive effects.

Experimental, as opposed to clinical, data in support of a role for *C. albicans* or components thereof in immunomodulation, have been generated in both *in vitro* lymphoproliferative[360–369] or antibody generating assays,[365,370,371] and in *in vivo* systems.[372–379] The *in vivo* studies were designed to investigate the immunomodulatory effects of various cell wall extracts on both *Candida*-specific[376,379,380] and noncandidal immune responses.[372–375,377,378]

Two types of *in vitro* assays have been used to demonstrate immuno-

regulatory phenomena associated with *C. albicans* or extracts thereof. First, human lymphocytes have been employed in lymphocyte stimulation (LS) assays,[366,381] and secondly, murine lymphoid cells have been used in LS or antibody plaque-forming cell (PFC) assays.[364,365] The assays with the human lymphocytes have been difficult to interpret in some instances because many humans have cellular immunity specific for *C. albicans*, and frequently investigators did not determine the underlying sensitivity patterns of their patients. For example, Piccolella *et al.*[381] reported on the "mitogenic" effect of an extract designated MPPS (Mangion purified polysaccharide) for human lymphocytes, which was in all likelihood *Candida*-specific stimulation. With that caveat in mind, however, using the human lymphocyte-MPPS system, the generation of suppressor cells was demonstrated,[363] as well as the production of a nonspecific inhibitor, which blocked interleukin-1 and depressed interleukin-2 production in 5–6-day cultures.[382]

In vivo and *in vitro* murine assays used for investigating immunoregulatory phenomena associated with *C. albicans* or its components are summarized in Table IV. The *in vitro* studies, done initially with formalin-killed cells as modulators, and later with a dithiothreitol cell wall extract, have resulted in the identification of both suppressor B lymphocytes[364,365] and antigen-nonspecific suppressor T lymphocytes.[370,371] The suppressor T cells were capable of inhibiting both primary and secondary *in vitro* antibody responses to the T-dependent antigen sheep erythrocytes (SRBC), and the lymphocytes were determined to be L3T4+, Ly-1+ and Lyt-2−. The antibody response to T-independent antigens, trinitrophenyl-LPS (type I) or trinitrophenyl-Ficoll (type II), were unaffected by the suppressor cell.

The *in vivo* studies have been targeted to investigations of immunoregulatory phenomena associated with both *Candida*-specific and non-*Candida*-specific responses. The initial studies were with whole fungal cells predominantly involving evaluations of nonspecific activity against T-cell dependent antigens or mitogens. Conflicting data were observed, in that Vardinon and Segal[373] noted suppression of anti-SRBC antibody responses, whereas Cutler and Lloyd[375] noted enhancement. Only suppression was observed with respect to T cell mitogens.[364] When soluble extracts were employed, enhancement of antibody responses to noncandidal antigens was noted in all cases where the extracts were glucomannoprotein complexes,[375,378] but when cell wall extracts devoid of the glucan component, i.e., mannan or mannoprotein,[383] were tested, antibody responses were either enhanced or suppressed.[377] The specific type of modulation observed was dependent on the size and charge of the component molecules within the mannan extract. Podzorski *et al.*[368] and Podzorski *et al.*[369] have obtained similar data *in vitro* with human lymphocytes and mannan extracts, i.e., extracts containing saccharides with > 7 saccharide units were stimulatory to lymphocytes from *C. albicans*-sensitized individuals, whereas oligosaccharides with ≤ 6 mannose residues were suppressive.

TABLE IV
Murine Model Systems used for the Demonstration of the Immunodulatory Capacity of *C. albicans* or Components thereof

Modulator	Extraction	Assay	Antigen/mitogen	Effect
In vivo models				
Live cells[373]	N/A	PFC	SRBC	Suppression
Live or killed[375] cells, cell walls, wall polysaccharide	N/A Phenol	PFC	SRBC, CGG-HE	Enhancement in all cases
Formalin-killed[364] cells	N/A	LS	PHA	Suppression
			Con A	Suppression
			LPS	No effect
Cell-wall glycoprotein[376]	Ethylenediamine	DH	Cell-wall glycoprotein	Suppression
Mannan[377]	Peat method	PFC	SSS-III and SRBC	Enhancement and suppression to both antigens
Cell-wall glycoprotein, and peptidoglucomannan[378]	Ethylenediamine NaOH	PFC	SSS-III and SRBC	Enhancement in all cases
Mannan[379]	Peat method	DH	Mannan	Suppression
In vitro models				
Formalin-killed cells[365]	N/A	LS	PHA	Suppression
			LPS	No effect
		PFC	SRBC	Suppression
Formalin-killed cells[370]	N/A	PFC	SRBC	Suppression
			TNP-LPS	No effect
			TNP-Ficoll	No effect
		MLR		No effect
Cell-wall extract[371]	Dithiothreitiol	PFC	SRBC	Suppression

Abbreviations: N/A, not applicable; PFC, antibody plaque-forming cell assay; SRBC, sheep erythrocytes; CGG-HE, chicken gamma globulin-horse erythrocytes; LS, lymphocyte stimulation; PHA, phytohemagglutinin; Con A, concanavalin A; LPS, lipopolysaccharide; DH, delayed hypersensitivity; SSS-III, pneumococcal polysaccharide type III; TNP-LPS, trinitrophenyllipopolysaccharide; TNP-Ficoll, trinitrophenyl-Ficoll; MLR, mixed lymphocyte reaction.

When soluble extracts, specifically glucomannoprotein[376] or mannoprotein,[379] were investigated for their potential to modulate *C. albicans*-specific cellular immunity, mannan-specific responses were suppressed but immune responses detectable with antigens that did not contain mannan were unaffected. The cell responsible for mannan-specific suppression is Thy 1.2+, Ly-1−, and Ly2.1+.[380] Since mannan has been shown to bind to human T lymphocytes,[384] binding of the polysaccharide to murine T cells may initiate the suppressor cell phenomenon. Mannan may actually exert its effect at several levels of the immune response, however. For example, Fischer *et al.*[385] have shown defective handling of mannan by MN, and Wright *et al.*[386] demonstrated inhibitory effects of mannan on PMNL function.

In summary, there is good evidence that subcellular components of *C. albicans* circulate during bouts of serious candidiasis and that suppression of *Candida*-specific and, occasionally, noncandidal immune responses occurs. The nature of the subcellular component may vary, but mannan is a good candidate for at least some of the immunosuppressive activity noted. Mannan can be immunosuppressive in experimental models, depending on the size and charge of the molecules involved, but it can also enhance immune responses, as can all cell wall extracts known to contain glucan as well as mannan tested to date. The mechanisms responsible for suppression have been identified as B and T lymphocytes, depending on the modulator and assay conditions but may also involve mononuclear cells and PMNL. Absolute correlation between the phenomena observed during clinical disease and those observed in model systems cannot be made at this time; additional data from both humans and animal models are needed.

2.3. Immunodiagnosis and Prognosis

The value of serology in the diagnosis of systemic candidiasis remains an enigma, despite the accumulation of 30 years of data on antibody responses and antigen detection. It was recognized early that both colonized and infected individuals regularly produced antibody, i.e., agglutinins, to surface immunogens of *C. albicans*.[387] Since it appeared that agglutinin production would be of little value in separating colonized individuals from those with systemic disease, Taschdjian *et al.*[388] proposed that cytoplasmic antigens would be released from phagocytic cells following ingestion and killing or after senescent extracellular cells died in deep tissues. Considerable effort has been expended, therefore, on attempting to diagnose invasive disease by detecting anticytoplasmic antibodies. Unfortunately, it has proven difficult to correlate cytoplasm-specific antibody levels with disease state, and the search for the unequivocal serological test for the diagnosis of systemic candidiasis continues.

There have been a number of reviews in recent years devoted to, or including, serodiagnosis of candidiasis,[5,17,389–391] some of which contain comprehensive coverage of the historical aspects of the development and

application of various immunologic assays. More recently, emphasis has been placed on qualitative rather than quantitative antibody assays and on antigen detection; these aspects of candidal serology will be emphasized below.

In order to develop a definitive test for the serodiagnosis of candidiasis, the investigator must keep several aspects of the disease firmly in mind:

1. Although there are many divisions into which one can place the clinical manifestations of candidiasis, basically there are two broad categories, i.e., cutaneous/mucocutaneous and systemic, and any serodiagnostic technique developed must distinguish between systemic and more superficial forms of the disease.
2. In both types of disease the immune system is exposed to several different forms of the fungus, namely, blastospores (yeasts), pseudohyphae, and hyphae. There is good evidence to suggest that antigens unique to blastospores or hyphae occur at both the cytoplasmic[392–394] and cell wall levels.[395–398] and that the expression of surface antigens is variable under various conditions of growth[399–401] as well as during morphogenesis.[400] Moreover, it was shown many years ago that at least two serotypes of *C. albicans* occur based on the surface antigen, mannan,[37,402] and since *C. albicans* serotype A is not the only *Candida* spp. capable of causing disease, there are concerns that serodiagnostic tests developed with *C. albicans* serotype A may not be applicable to all *Candida* spp. Therefore, not only is the selection of antigen for the serodiagnostic test critical, but the time during the course of disease at which sera are obtained from patients is also critical.
3. The patients from whom sera are being obtained for serodiagnostic tests are frequently immunosuppressed and may have low or nonexistent levels of antibody.
4. The results of many studies in which investigators were attempting to determine the validity of cytoplasmic rather than cell wall extracts as antigens are called into question by the observation that cytoplasmic extracts, whether commercially obtained or prepared in-house, often contain mannan.[403,404]
5. The interpretation of data from many studies is complicated because each investigator tends to use his or her own definition of clinical conditions. For example, if candidemia, a condition that may or may not lead to systemic disease, is included in the category of systemic candidiasis, the conclusions drawn from the data may be quite different than if candidemia is considered separately from truly invasive disease.

2.3.1. Antibody Assays

A number of different techniques have been employed to detect anticytoplasmic antibodies in the sera of patients suspected of having, or with proven, systemic candidiasis, including double immunodiffusion (ID) and

latex agglutination (LA),[405] counterimmunoelectrophoresis (CIE),[406] crossed immunoelectrophoresis (XIE),[407] and enzyme immunoassay (ELISA).[408,409] Odds[17] catalogued the known studies up to 1988. It is clear that most healthy individuals do not have antibodies to cytoplasmic antigens, even when a particularly sensitive assay such as ELISA was used. To the contrary, many individuals with other diseases, fungal and nonfungal, have antibodies detectable by each of these assays, the highest number of false negatives occurring when the LA assay was used. The assay showing the least false negatives was XIE; it is also the most difficult of the assays listed to perform and adapt to the routine diagnostic laboratory.

The one assay that has probably been most helpful as a diagnostic aid has been CIE.[410–416] Therefore, several laboratories have developed modifications of this technique in attempts to improve its usefulness further. First, Syverson *et al.*[417] reported that the positive predictive value could be increased from 31% to 71% when using XIE. This is a technique involving electrophoresis in two directions. The antigenic mixture is separated in one direction first, then electrophoresed through two additional gels in sequence; the first contains con A to remove mannan and the second contains the patient's serum. Additional studies with this technique supported the claim that if precipitins were detected in the sera of patients after its passage through con A, those patients had high likelihoods of having systemic candidiasis.[418]

A second modification of CIE, designated co-CIE, which has proven useful in some hands[419,420] but not others,[421] was developed in France. This assay involves the demonstration of lines of identity between precipitin reactions resulting from positive control antisera and selected antigenic extracts and those resulting from patient sera tested against the same extracts. The precipitin reaction which appeared most promising is that between a "somatic" antigen, i.e, a soluble antigen prepared as a water extract of whole disrupted cells, and an antiserum generated against germ tubes of *C. albicans*. Unfortunately, in the studies designed to evaluate this technique, a distinction was not made between candidal septicemia and true systemic candidiasis and the value of this technique as a diagnostic and prognostic aid remains to be confirmed.

Because of the difficulty in interpretation of the serologic assays available commercially as kits or for which component reagents are available, one of the major emphases in the last ten years has been on attempting to identify one or more immunodominant antigens to which all or most individuals with systemic candidiasis, but not with superficial colonization or candidemia, would produce antibody. To demonstrate such antigens, most investigators have used immunoblotting, i.e, the Western blotting technique, wherein candidal antigens are electrophoresed in agarose gel and then incubated in patient sera. Reactions between patient sera and selected antigens in the electrophoretic profile are detected by radioimmunoassay or enzyme-linked

immunoassay. Jones[422] was the first to report on a candidate antigen of this type in yeast extracts, and several additional groups subsequently reported similar findings in either yeast[423–425] or mycelial[425,426] extracts. There seems to be general agreement among these five investigative groups that most patients with systemic candidiasis produce antibody to an antigen in the 43–54 kDa range, and Porsius *et al.*[427] recently reported that intense reactions to a 47 kDa and a 68 kDa antigen were more often associated with sera from 15 patients with disseminated candidiasis than with sera from 12 individuals who were only colonized. It is not clear whether or not each group of investigators has identified the same antigen in the 43–54 kDa range since no comparative studies have been reported, although the data obtained by Strockbine *et al.*[426] would seem to indicate that their 48 kDa antigen is different from the 54 kDa antigen of Greenfield and Jones.[428] Strockbine *et al.*[426] isolated their antigen from mycelial cells, whereas Greenfield and Jones[428] isolated theirs from yeast cells.

Greenfield and Jones[428] purified their major cytoplasmic antigen, the source of which was yeast cells, using column chromatography with an ion exchange resin followed by con A-sepharose. The purified protein had a molecular weight of 54,300, had 432 amino acid residues, was electrophoretically pure, but had some low level contaminating proteins demonstrable by XIE. When tested for enzymic activity, it was inactive against 19 substrates for common cellular enzymes. Strockbine *et al.*[426] have been attempting to characterize their 48 kDa antigen as well. Initially, it was thought to be actin because preparations of the 48 kDa antigen obtained contained actin. When purified actin was compared to the 48 kDa antigen, however, it was clear that the two proteins were not identical.[429] Subsequently, they demonstrated that the protein was enolase.[430] In support of the enolase data, Franklyn *et al.*[431] prepared cDNA from *C. albicans* yeasts which encoded a portion of the 48 kDa antigen and determined from the deduced amino acid sequence that the 48 kDa protein was an enolase. The 47 kDa antigen of Matthews *et al.*[432] was isolated from patient sera by affinity chromatography using rabbit antisera prepared against a cell homogenate, and by using affinity-purified antibody and immunoelectron microscopy, Matthews *et al.*[433] located the 47 kDa antigen in selected areas within the cell wall and in the peripheral aspects of the cytoplasm.

Despite the studies suggesting a correlation between production of antibody to specific antigens and disease classification, there are reports from other laboratories that are not in agreement with that hypothesis. Manning-Zweerink *et al.*,[434] Reen,[435] and Weller *et al.*,[436] for example, were unable to correlate qualitative antibody responses with clinical condition. In fact, Weller *et al.*[436] observed that both controls and patients made antibody to the 47 kDa antigen. Their primary objective was to evaluate sera from individuals with superficial candidiasis, and they concluded that such individuals responded to antigens of 29, 32, 38, and 65 kDa. Moreover, Greenfield *et al.*,[437] following

a series of patients undergoing induction chemotherapy for acute leukemia, found few instances of antibody to their 54 kDa antigen during episodes of invasive candidiasis. It is clear that the identification of immunodominant antigens and their diagnostic and prognostic value for systemic candidiasis remains controversial, although it does hold some promise for the future, perhaps as antigen to which antibodies can be generated for detection of antigenemia.[438]

It has been suggested that antibodies to germ tube antigens may be more diagnostic of active disease than those specific for yeasts only, and an indirect immunofluorescence assay was developed for that specific purpose.[439] Antibodies specific for the yeast phase must be removed from specimens under consideration before testing for the germ tube-specific antibodies. Quindos *et al.*[440,441] used this assay in a retrospective study of various groups of patients and concluded that the test was able to discriminate between systemic candidiasis and other invasive mycoses. Its usefulness as a diagnostic aid for detection of systemic candidiasis remains to be determined in prospective studies including colonized patients, as well as those with superficial disease.

Finally, a novel assay has been described for the detection of antibodies to different antigens in a single assay, the usefulness of which is unknown at this stage, but it has potential for future development. McHugh *et al.*[442] coated polystyrene beads of varying diameters with three separate antigenic preparations of *C. albicans*, namely, a whole cell extract, a cytoplasmic extract and a cell wall polysaccharide, and tested patient sera in an assay that allowed for the separation of beads by flow cytometry. There are all sorts of possibilities for alterations in the protocol presented that could ultimately lead to a very specific assay for diagnosis of systemic candidiasis based on antibody responses to specific antigens.

It appears to be the consensus of those most heavily involved in serodiagnostic assay performance and development that antibody detected in any one of these assays, when evaluated in the context of the patient's clinical condition and other laboratory parameters, such as culture, can be helpful diagnostically and prognostically, but when evaluating only a single specimen, antibody data generated by these techniques are nearly impossible to interpret.

2.3.2. Antigen Detection

Early diagnosis of systemic candidiasis is necessary if effective therapy is to be instituted. It is clear that detection of antibody has not provided appropriate assays for early diagnosis of this clinical condition, and it was reasoned that detection of antigen might provide more rapid and definitive diagnostic data. Emphasis for antigen detection has been with two types of preparations, namely, antimannan antibody and antibody directed against a heat-labile antigen(s) that does not appear to be mannan. The first antigen targeted for detection was mannan, and hemagglutination inhibition,[443]

CIE,[444] radioimmunoassay,[445] ELISA-inhibition,[409,446] and ELISA[447] assays were developed for trials. In the original study,[443] only 4 of 14 individuals with proven systemic candidiasis had demonstrable mannan antigenemia. Kerkering *et al.*[444] had somewhat better results, in that eight out of 13 patients who eventually developed systemic candidiasis were positive. In yet another study, only five of 11 patients with systemic candidiasis had circulating mannan detectable by RIA.[445]

Since mannan can be present in sera in immune complexes, several investigators have suggested dissociative methods[447] or heat extraction[448] for increasing the likelihood of mannan detection. Although investigators do use these dissociative steps,[449,450] it is not clear that the sensitivity of the test is greatly increased. Others[450,451] have suggested that serial assays for mannan are important, perhaps because mannan has a relatively short half-life in serum[422] and is not released continuously from sites of infection.[450] There is a single mannan-detection kit available commercially in the United States, the LA-*Candida* Antigen Detection System, Immuno-Mycologics, Inc., Norman, Oklahoma. That kit appears to be relatively insensitive in that none of the patients tested by Phillips *et al.*,[452] which included 33 patients with candidemia, none of those tested by Fung *et al.*,[453] which included 9 patients with invasive candidiasis, and none tested by Bisbe *et al.*,[416] which included 36 heroin addicts diagnosed as having systemic candidiasis, were positive. Many of those same patients tested for a putatively different antigen (see below) were positive. Several groups[449,454] have detected mannan by ELISA with considerably more success in terms of higher titers, but increasing the sensitivity decreased the specificity with regard to invasive candidiasis and its distinction from colonization. For example, Fujita *et al.*[454] detected mannan in nine of 10 patients with systemic candidiasis, but it was also detected in two of four patients who had superficial candidiasis. Fujita *et al.*[454] correlated their antigenemia studies with observations of antibody; antigenemia preceded rising antibody titers by 6 to 23 days.

A second antigen detection system, originally developed by Gentry *et al.*[455] and now marketed as the CAND-TEC™ by Ramco Laboratories, Inc., Houston, Texas, has been evaluated rather extensively by several different laboratories.[449,452,453,456–458] In one study,[452] only patients with candidemia were evaluated, along with patients at risk and healthy subjects, but in all others,[449,453,456–458] patients with proven invasive candidiasis were included. Piens *et al.*,[456] Ness *et al.*,[457] and Escuro *et al.*[458] determined the sensitivity to be 38%, 55%, and 76%, the specificity to be 90%, 29%, and 93%, the positive predictive value to be 50%, 17%, and 50%, and the negative predictive value to be 85%, 71%, and 100%, respectively.

The data available suggest that when using the CAND-TEC system, a titer of $\geq$ 1:4 is equivocal and $\geq$ 1:8 is evidence of disseminated disease.[453,455] If that criterion was applied, sensitivity in the data reported by Piens *et al.*[456] would fall to 0%, since none of their patients had titers > 1:4. In a comparative study of the CAND-TEC and ELISA assays, only four of 14 patients with

disseminated candidiasis were positive and 4/22 patients were positive by ELISA using 1:8 as the cutoff point. Others have suggested that rheumatoid factor[449] and high creatinine levels[457] interfere with the test, but Price and Gentry[459] found no interference with the test in patients undergoing dialysis, and they[460] determined in their survey that patients with rheumatoid factor and no antigen had titers of $\leq$ 1:2. The latter investigators, as well as others,[450] suggested also that the testing of sequential specimens would increase the likelihood of detecting antigen, but Escuro *et al.*,[458] using weekly sampling, were not able to improve their detection rate. Pretreatment of serum with protease and heat improved results here as well.[461] One clinical condition where the CAND-TEC system would appear to be very valuable is in the diagnosis of pulmonary candidiasis,[462] a condition that, in general, is very difficult to diagnose. Fifteen of 16 patients with clinical and laboratory evidence of *C. albicans* pneumonia had positive CAND-TEC titers when bronchoalveolar lavage fluids were tested.

At least two additional antigen detection systems have been described but neither appears to have been tested in other laboratories, and their potential as candidate tests for general use is unknown.[463],[464] Moreover, Western blotting has been used recently to detect candiduria in patients with multiple positive blood cultures.[465] The results of this one study suggest that detection of antigenuria may be useful diagnostically and prognostically, but more data are clearly needed to confirm the hypothesis.

2.3.3. Summary

It is obvious from the above discussion that the development of serodiagnostic tests for the diagnosis and prognosis of systemic candidiasis is a high priority in a number of laboratories. It is also obvious that no one test has yet been devised that is appropriately sensitive and specific for application to the routine clinical laboratory. For those laboratories equipped to do it, CIE or XIE appear the most reliable antibody assays, whereas an ELISA assay for mannan appears to be the most sensitive for antigen detection. As stated by Odds,[17] however, "[Serodiagnosis] is one of the fastest developing aspects of candidal research. Those who devise novel serological approaches to diagnosis usually present their work in a positive fashion: those who apply them in clinical practice are usually more or less disappointed."

2.3.4. Cell-Mediated Immunity (CMI)

2.3.4a. CMI in Humans. A high percentage of normal individuals, ranging from 54 to 94%, depending on the survey,[466–470] have DH demonstrable by skin testing with *C. albicans* antigens. In one of the more extensive studies, Shannon *et al.*[467] noted 80%, 89%, and 83% positive rates for children 7–12 months of age, 1–5 years of age, and 5–15 years of age, respectively, and 94%

in adults. Because of the high response rate in the general population, skin testing is of little value for the diagnosis or prognosis of systemic candidiasis, although skin test response and response rates in *in vitro* cell-mediated assays have been used to categorize patients with chronic mucocutaneous candidiasis.[471]

Since there is such a high rate of responders in the population-at-large, a *C. albicans* extract is usually included in the battery of common recall antigens administered to patients in whom immunodeficiency is suspected. The antigen most frequently employed is a poorly defined *C. albicans* extract obtained from Hollister-Stier (Spokane, WA). Caution should be exercised in its use if applied repeatedly to the same patient, however, in that Hogan *et al.*[470] have noted increased responsiveness after sequential skin test applications. It would be ideal to have available a reliable *in vitro* assay that could be employed to determine cellular immunity to *C. albicans*, and to that end, several groups of investigators have compared skin test results in normal patients with *in vitro* correlations of DH such as LS[469,472] and migration inhibition assay.[468,472] Close correlations were found between skin test reactivity and LS assays, but contradictory data were obtained using the migration inhibition assay.

The Hollister-Stier antigen has been used for LS *in vitro*,[469,473] and other investigators have used other commercial antigens[472] or extracts prepared in the laboratory.[474] Most of these extracts are poorly defined complex mixtures. The development of new antigenic preparations for *in vivo* or *in vitro* testing has moved quite slowly, in fact, although Esch and Buckley[475] reported several years ago on a new type of extract they tested in humans. It was an ammonium sulfate fractionation of an aqueous extract of lyophilized *C. albicans* yeast. Fifty percent of a cohort of the normal population responded with reactions of $\geqslant$ 5 mm at 48h when tested with 1 μg of the preparation.

2.3.3b. CMI in Experimental Models. Several different preparations, including heat-killed cells,[476,477] cell wall extracts,[379,478–482] and cytoplasmic components[481,483] have been used in animal models in attempts to develop skin test antigens or correlate DH with other immune responses in animals. Evaluation of these antigens as potential candidates for human use has been complicated by the fact that, in a number of instances, the antigens were tested in animals immunized with nonviable extracts rather than infected with viable organisms.[478–480,483] Further, skin test results with particulate antigens, as when heat-killed cells are employed, are difficult to interpret because of the possibility of B lymphocytes producing antibody *in situ*,[484] and/or foreign body reactions resulting in complement activation, the latter of which could result in enhanced antigen retention and slowed antigen degradation. Moreover, in one human study,[485] the injection of whole cells elicited a strong eosinophil response that persisted through 24h.

Cell wall and membrane components, as opposed to cytoplasmic preparations, appear to be the best antigens for *in vivo* testing described to date in

infected animals.[379,479,481,482] Several types of cell wall preparations have been used, ranging from glycoproteins extracted with ethylenediamine (EDA)[481,482] and cold dilute alkali,[479] to mannans extracted by the Peat *et al.* method.[379,383] Domer and Moser[481] compared many of these extracts, cell wall and cytoplasmic, the latter of which included both ribosomes and soluble cytoplasmic substances, in a murine model. A membrane extract and several cell wall preparations elicited the best responses. Many responses, however, were complicated by large 4h reactions. In subsequent studies, however, it was possible to demonstrate that if the cell wall glycoprotein extracted with EDA was subjected to extensive dialysis with 1 M NaCl to ensure removal of all ethylenediamine,[379] it no longer elicited the large early responses. Moreover, testing of infected mice with mannan extracted by the Peat *et al.* method[383] did not result in large early responses either, although such responses had been demonstrated in guinea pigs.[480] The lack of early response in infected mice may reflect the fact that mice respond poorly to mannan with respect to antibody production.[486] The response to the EDA-extracted glycoprotein was destroyed by treatment with proteolytic enzymes and partially ablated by periodate oxidation of the preparation.[481] Moreover, the *in vivo* responses to that glycoprotein, as well as to a membrane preparation extracted with hot phosphate-buffered saline, could be transferred with T lymphocytes.[481,482]

Many of the preparations used for *in vivo* testing have been tested for their applicability in *in vitro* tests, especially for LS assays. Moser *et al.*,[482] for example, compared EDA-extracted cell wall glycoprotein, a soluble membrane extract, and soluble cytoplasmic substances, for their ability to stimulate lymphocytes from infection mice. Responses to the cell wall and membrane extracts correlated well with DH responses *in vivo*, but surprisingly, the cytoplasmic preparation, a preparation which was poorly reactive *in vivo*, induced strong proliferative responses *in vitro*. These studies confirmed an earlier study with human cells in which cytoplasmic and cell wall antigens were compared, in that the EDA-extracted material elicited the best proliferative responses.[474] Although the EDA-extracted glycoprotein induced lymphocytes to proliferate *in vitro*,[482,487] as did a phosphorylated mannoprotein complex from the cell wall of *C. albicans*[488] and an ethanol-precipitated and deproteinized extract,[489] mannan did so poorly, if at all, with human[488,490,491] or murine lymphocytes.[487] Tollemar *et al.*[491] noted a much better response in peripheral blood lymphocytes to cytoplasmic antigens, as did Gettner and Mackenzie.[490] The conflict between the *in vivo* and *in vitro* data with mannan, may relate to the extraction procedure. Most investigators have used the method of Peat *et al.*[383] Podzorski *et al.*[369] tested a mannan extracted with cetryltrimethylammonium bromide and found it to be a potent stimulator of *in vitro* lymphoproliferation for human lymphocytes. Small oligosaccharides derived from that mannan were not stimulatory, however. Perhaps smaller molecular weight components in the Peat *et al.*[383] extract act to depress the response *in vitro* but not *in vivo*.

2.3.4c. Summary. It is clear from many studies that a high percentage of humans develop cellular immunity demonstrable *in vivo* by skin testing and *in vitro* by testing for proliferation of lymphocytes when testing is done with a number of different antigenic mixtures. It is also quite clear that detection of cellular immune responses is hampered by a lack of standardization in methodology and in the antigenic preparations employed. The preparations available commercially are poorly defined complex mixtures, and those developed in the laboratory and tested under experimental conditions have not yet been tested in prospective studies with normal subjects and human patients with various forms of candidiasis. It is difficult, in fact, based on the experimental work, to make generalizations as to which of the many preparations tested should be targeted for development for *in vivo* or *in vitro* testing, although it would appear that mannan should not be considered. Other cell wall antigens appear more promising, but since many normal individuals have circulating antibody to cell wall antigens, large, immediate hypersensitivity reactions might be expected with cell wall antigens.

2.4. Therapy of Systemic Candidiasis

The treatment of systemic candidiasis generally consists of measures directed against the underlying condition(s) (e.g., neutropenia or leukemia) and administration of an antifungal agent. Because difficulties are often encountered in establishing the diagnosis and because the infection often occurs in complex clinical settings, clinicians must be guided by the "art" of medicine, as well as its "literature," in deciding when to initiate antifungal treatment, what to use, and how long to continue the therapy.

Amphotericin B, a polyene antibiotic produced by *Streptomyces nodosus*, is the current mainstay of therapy. Amphotericin B interacts with fungal plasma membrane sterols and renders the membrane leaky.[492] Resistant *Candida* spp. organisms are uncommon but have been described. Because amphotericin B is insoluble, the intravenous form of this drug is prepared as a complex with deoxycholate. The intravenous administration of amphotericin B-deoxycholate is associated with considerable host toxicity and regularly causes fever, chills, impaired renal function, and anemia. Bennett[493] has provided excellent guidelines for its clinical use. Considerable interest attends the development of alternative, less toxic liposomal formulations of amphotericin B, some of which are in current clinical trials.

When used in combination with amphotericin B, 5-fluorocytosine may have utility in treating candidal meningitis.[494] This agent is converted by deamination and phosphorylation to 5-fluorodeoxyuridylic acid, a competitive inhibitor of thymidylate synthetase,[495] and is also incorporated extensively into fungal RNA.[496] Primary resistance of *C. albicans* to the agent is not uncommon.[496]

Imidazoles (e.g. ketoconazole) and the closely related triazoles (e.g.,

fluconazole) inhibit fungal ergosterol production by inhibiting cytochrome P_{450}-dependent 14-α-demethylase.[497] The drugs are effective after oral administration and are generally much less toxic than amphotericin B-deoxycholate complex. Fluconazole attains excellent penetration into the cerebrospinal fluid.[498] Although it has given promising results in the treatment of oropharyngeal candidiasis in patients with cancer[499] and AIDS,[500] its efficacy in systemic candidiasis is unproven. Perhaps, as with the earlier imidazole drug ketoconazole,[501–504] its primary utility will be in chemoprophylaxis.

For those seeking a broader description of the clinical syndromes of systemic candidiasis and their treatments, the excellent recent reviews by Edwards[504] and Meunier[505] are highly recommended.

ACKNOWLEDGMENTS. This work was supported in part by Public Health Service grants AI-12806 and AI-22839 from the National Institute of Allergy and Infectious Diseases. We thank Ersebet Huffman and Sally Grogono for expert assistance in manuscript preparation.

This chapter was completed in January 1991.

REFERENCES

1. Cohen, R., Roth, F. J., Delgado, E., Ahearn, D. G., and Kalser, M. H., 1969, Fungal flora of the normal human small and large intestine, *N. Engl. J. Med.* **280:**638–641.
2. Draser, B. S., and Hill, M. J., 1974, *Human Intestinal Flora*, Academic Press, New York.
3. Kennedy, M. J., 1989, Regulation of *Candida albicans* populations in the gastrointestinal tract: mechanisms and significance in GI and systemic candidiasis, *Curr. Top. Med. Mycol.* **3:** 315–402.
4. Budtz-Jorgensen, E., 1990, Etiology, pathogenesis, therapy, and prophylaxis of oral yeast infections, *Acta Odontol. Scand.* **48:**61–69.
5. Domer, J. E., and Carrow, E. W., 1989, Candidiasis, in: *Immunology of the Fungal Diseases* (R. A. Cox, ed.), CRC Press, Inc., Boca Raton, Florida, pp. 57–91.
6. Young, R. C., Bennett, J. E., Geelhoed, G. W., and Levine, A. S., 1974, Fungemia with compromised host resistance. A study of 70 cases, *Ann. Intern. Med.* **80:**605–612.
7. Parker, J. C., Jr., McCloskey, J. J., and Knauer, K. A., 1976, Pathobiologic features of human candidiasis. A common deep mycosis of the brain, heart and kidney in the altered host, *Am. J. Clin. Pathol.* **65:**991–1000.
8. Horn, R., Wong, B., Kiehn, T. E., and Armstrong, D., 1985, Fungemia in a cancer hospital: changing frequency, earlier onset and results of therapy, *Rev. Infect. Dis.* **7:**646–655.
9. Pirsch, J. D., and Maki, D. G., 1986, Infectious complications in adults with bone marrow transplantation and T-cell depletion of donor marrow, *Ann. Intern. Med.* **104:**619–631.
10. Tollemar, J., Ringden, O., Bostrom, L., Nilsson, B., and Sundberg, B., 1989, Variables predicting deep fungal infections in bone marrow transplant recipients, *Bone Marrow Transplantation* **4:**635–641.
11. Tapper-Jones, L., Aldred, M. J., Walker, D. M., and Hayes, T. M., 1981, Candidal infections and populations of *Candida albicans* in the mouths of diabetics, *J. Clin. Pathol.* **34:**706–711.
12. Prasad, J. K., Feller, I., and Thomson, P. D., 1987, A ten-year review of *Candida* sepsis and mortality in burn patients, *Surgery* **101:**213–216.
13. Ryley, J. F., 1986, Pathogenicity of *Candida albicans* with particular reference to the vagina, *J. Med. Vet. Mycol.* **24:**5–22.

14. Montgomerie, J. Z., and Edwards, J. E., Jr., 1978, Association of infection due to *Candida albicans* with intravenous hyperalimentation, *J. Infect. Dis.* **137:**197–201.
15. Karabinis, A., Hill, C., Leclercq, B., Tancrede, C., Baume, D., and Andremont, A., 1988, Risk factors for candidemia in cancer patients: a case-control study, *J. Clin. Microbiol.* **26:** 429–432.
16. Bross, J., Talbot, G. H., Maislin, G., Hurwitz, S., and Strom, B. L., 1989, Risk factors for nosocomial candidemia: a case-control study in adults without leukemia, *Am. J. Med.* **87:** 614–620.
17. Odds, F. C., 1988, *Candida and Candidosis*, Bailliere Tindall, London, pp. 93–114.
18. Pfaller, M. A., 1988, Nosocomial *Candida* infections, *Curr. Opin. Infect. Dis.* **1:**764–771.
19. Rippon, J. W., 1988, *Medical Mycology, The Pathogenic Fungi and the Pathogenic Actinomycetes*, W. B. Saunders Company, Philadelphia, PA, pp. 532–581.
20. Spencer, P. M., and Jackson, G. G., 1989, Fungal and mycobacterial infections in patients infected with the human immunodeficiency virus, *J. Antimicrob. Chemother.* **23**(suppl. A)**:** 107–125.
21. Klein, R. S., Harris, C. A., Small, C. B., Moll, B., Lesser, M., and Friedland, G. H., 1984, Oral candidiasis in high-risk patients as the initial manifestation of the acquired immunodeficiency syndrome, *N. Engl. J. Med.* **311:**354–358.
22. Tavitian, A., Raufman, J.-P., and Rosenthal, L. E., 1986, Oral candidiasis as a marker for esophageal candidiasis in the acquired immunodeficiency syndrome, *Ann. Intern. Med.* **104:** 54–55.
23. Guentzel, M. N., Cole, G. T., and Pope, L. M., 1985, Animal models for candidiasis, *Curr. Top. Med. Mycol.* **1:**57–116.
24. Kreger-van Rij, N. J. W., 1984, *The Yeasts. A Taxonomic Study*, Elsevier Science Publishers B. V., Amsterdam, pp. 585–844.
25. Kiehn, T. E., Edwards, F. F., and Armstrong, D., 1980, The prevalence of yeasts in clinical specimens from cancer patients, *Am. J. Clin. Path.* **73:**518–521.
26. Wingard, J. R., Merz, W. G., and Saral, R., 1979, *Candida tropicalis*: a major pathogen in immunocompromised patients, *Ann. Intern. Med.* **91:**539–543.
27. Meunier-Carpentier, F., Kiehn, T. E., and Armstrong, D., 1981, Fungemia in the immunocompromised host. Changing patterns, antigenemia, high mortality, *Am. J. Med.* **71:**363–370.
28. Walsh, T. J., and Merz, W. G., 1986, Pathologic features in the human alimentary tract associated with invasiveness of *Candida tropicalis*, *Am. J. Clin. Pathol.* **85:**498–502.
29. Howlett, J. A., 1976, The infection of rat tongue mucosa *in vitro* with five species of *Candida*, *J. Med. Microbiol.* **9:**309–316.
30. Allen, C. M., and Beck, F. M., 1987, Differences in mucosal reaction related to *Candida albicans* isolates, *J. Oral Pathol.* **16:**89–93.
31. Martin, M. V., Craig, G. T., and Lamb, D. J., 1984, An investigation of the role of true hypha production in the pathogenesis of experimental oral candidosis, *Sabouraudia* **22:**471–476.
32. 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:**576–580.
33. Walsh, T. J., and Pizzo, P. A., 1988, Nosocomial fungal infections: a classification for hospital-acquired fungal infections and mycoses arising from endogenous flora or reactivation, *Ann. Rev. Microbiol.* **42:**517–545.
34. Hadfield, T. L., Smith, M. B., Winn, R. E., Rinaldi, M. G., and Guerra, C., 1987, Mycoses caused by *Candida lusitaniae*, *Rev. Infect. Dis.* **9:**1006–1012.
35. Dick, J. D., Rosenguard, B. R., Merz, W. G., Stuart, R. K., Hutchins, G. M., and Saral, R., 1985, Fatal disseminated candidiasis due to amphotericin B-resistant *Candida guilliermondii*, *Ann. Intern. Med.* **102:**67–68.
36. Pappagianis, D., Collins, M. S., Hector, R., and Remington, J., 1979, Development of resistance to amphotericin B in *Candida lusitaniae* infecting a human, *Antimicrob. Agents Chemother.* **16:**123–126.

37. Hasenclever, H.F ., and Mitchell, W. O., 1961, Antigenic studies of *Candida*. I. Observations of two antigenic groups in *Candida albicans*, *J. Bacteriol.* **82:**570–573.
38. Odds, F. C., and Abbott, A. B., 1980, A simple system for the presumptive identification of *Candida albicans* and differentiation of strains within the species, *Sabouraudia* **18:**301–317.
39. Odds, F. C., and Abbott, A. B., 1983, Modification and extension of tests for differentiation of *Candida* species and strains, *Sabouraudia* **21:**79–81.
40. McCreight, M. C., and Warnock, D. W., 1982, Enhanced differentiation of isolates of *Candida albicans* using a modified resistogram method, *Mykosen* **25:**589–598.
41. Polonelli, L., Archibusacci, C., Sestito, M., and Morace, G., 1983, Killer system: a simple method for differentiating *Candida albicans* strains, *J. Clin. Microbiol.* **17:**774–780.
42. Roman, M. C., and Linares Sicilia, M. J., 1983, Preliminary investigation of *Candida albicans* biovars, *J. Clin. Microbiol.* **18:**430–431.
43. Lee, W., Burnie, J., and Matthews, R., 1986, Fingerprinting *Candida albicans*, *J. Immunol. Methods* **93:**177–182.
44. Phongpaichit, S., Mackenzie, D. W. R., and Fraser, C., 1987, Strain differentiation of *Candida albicans* by morphotyping, *Epidemiol. Infect.* **99:**421–428.
45. Magee, B. B., D'Souza, T. M., and Magee, P. T., 1987, Strain and species identification by restriction fragment length polymorphisms in the ribosomal DNA repeat of *Candida* species, *J. Bacteriol.* **169:**1639–1643.
46. Scherer, S., and Stevens, D. A., 1987, Application of DNA typing methods to epidemiology and taxonomy of *Candida* species, *J. Clin. Microbiol.* **25:**675–679.
47. Magee, B. B., and Magee, P. T., 1987, Electrophoretic karyotypes and chromosome numbers in *Candida* species, *J. Gen. Microbiol.* **133:**425–430.
48. Merz, W. G., Connelly, C., and Hieter, P., 1988, Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*, *J. Clin. Microbiol.* **26:**842–845.
49. Vaudry, W. L., Tierney, A. J., and Wenman, W. M., 1988, Investigation of a cluster of systemic *Candida albicans* infections in a neonatal intensive care unit, *J. Infect. Dis.* **158:**1375–1379.
50. Burnie, J. P., Odds, F. C., Lee, W., Webster, C., and Williams, J. D., 1985, Outbreak of systemic *Candida albicans* in intensive care unit caused by cross infection, *Brit. Med. J. (Clin. Res.)* **290:**746–748.
51. Odds, F. C., Palacio-Hernanz, A., Cuadra, J., and Sanchez, J., 1987, Disseminated *Candida* infection syndrome in heroin addicts—dominance of a single *Candida albicans* biotype, *J. Med. Microbiol.* **32:**275–277.
52. Neely, A. N., Odds, F. C., Basatia, B. K., and Holder, I. A., 1988, Characterization of *Candida* isolates from pediatric burn patients, *J. Clin. Microbiol.* **26:**1645–1649.
53. Fox, B. C., Mobley, H. L. T., and Wade, J. C., 1989, The use of a DNA probe for epidemiological studies of candidiasis in immunocompromised hosts, *J. Infect. Dis.* **159:** 488–494.
54. Pfaller, M., 1989, Infection control: opportunistic fungal infections—the increasing importance of *Candida* species, *Infect. Control Hosp. Epidemiol.* **10:**270–273.
55. Stevens, D. A., Odds, F. C., and Scherer, S., 1990, Application of DNA typing methods to *Candida albicans* epidemiology and correlations with phenotype, *Rev. Infect. Dis.* **12:** 258–266.
56. Soll, D. R., Staebell, M., Langtimm, C., Pfaller, M., Hicks, J., and Gopala Rao, T. V., 1988, Multiple *Candida* strains in the course of a single systemic infection, *J. Clin. Microbiol.* **26:** 1448–1459.
57. Burkholder, P. R., 1943, Vitamin deficiencies in yeasts, *Am. J. Botany* **30:**206–211.
58. Drouhet, E., and Vieu, M., 1957, Facteurs vitaminiques de croissance des *Candida*, *Ann. Inst. Pasteur* **92:**825–831.
59. Caroline, L., Taschdjian, C. L., Kozinn, P. J., and Schade, A. L., 1964, Reversal of serum fungistasis by addition of iron, *J. Invest. Dermatol.* **42:**415–419.

60. Masson, P. L., Heremans, J. F., and Schonne, E., 1969, Lactoferrin, an iron-binding protein in neutrophilic leukocytes, *J. Exp. Med.* **130:**643–658.
61. Bretz, U., and Baggiolini, M., 1974, Biochemical and morphological characterization of azurophil and specific granules of human neutrophilic polymorphonuclear leukocytes, *J. Cell Biol.* **63:**251–269.
62. Rado, T. A., Wei, X., and Benz, E. J., Jr., 1987, Isolation of lactoferrin cDNA from a human myeloid library and expression of mRNA during normal and leukemic myelopoiesis, *Blood* **70:**989–993.
63. Kirkpatrick, C. H., Green, I., Rich, R. R., and Schade, A. L., 1971, Inhibition of growth of *Candida albicans* by iron-unsaturated lactoferrin: relation to host-defense mechanisms in chronic mucocutaneous candidiasis, *J. Infect. Dis.* **124:**539–544.
64. McNamara, M. P., Wiessner, J. H., Collins-Lech, C., and Hahn, B. L., 1988, Neutrophil death as a defence mechanism against *Candida albicans* infections, *Lancet* **2:**1163–1165.
65. Sohnle, P. G., and Collins-Lech, C., 1990, Comparison of candidacidal and candidastatic activities of human neutrophils, *Infect. Immun.* **58:**2696–2698.
66. Steinbakk, M., Naess-Andresen, C.-F., Lingaas, E., Dale, I., Brandtzaeg, P., and Fagerhol, M. K., 1990, Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin, *Lancet* **336:**763–765.
67. Knight, L., and Fletcher, J., 1971, Growth of *Candida albicans* in saliva; stimulation by glucose associated with antibiotics, corticosteroids, and diabetes mellitus, *J. Infect. Dis.* **123:** 371–377.
68. Epstein, J. B., Truelove, E. L., and Izutzu, K. T., 1984, Oral candidiasis: pathogenesis and host defense, *Rev. Infect. Dis.* **6:**96–106.
69. Fisker, A. V., Schiott, C. R., and Philipsen, H. P., 1982, Short-term oral candidosis in rats, with special reference to the site of infection, *Acta Pathol. Microbiol. Immunol. Scand. B* **90:** 49–57.
70. Russell, C., Jones, J. H., and Gibbs, A. C. C., 1976, The carriage of *Candida albicans* in the mouths of rats treated with tetracycline briefly or for a prolonged period, *Mycopathologia* **58:**125–129.
71. DeMaria, A., Buckley, H., and von Lichtenberg, F., 1976, Gastrointestinal candidiasis in rats treated with antibiotics, cortisone and azathioprine, *Infect. Immun.* **13:**1761–1770.
72. Cantorna, M. T., and Balish, E., 1990, Mucosal and systemic candidiasis in congenitally immunodeficient mice, *Infect. Immun.* **58:**1093–1100.
73. Ouellette, A. J., Greco, R. M., James, M., Frederick, D., Naftilan, J., and Fallon, J. T., 1989, Developmental regulation of cryptdin, a corticostatin/defensin precursor mRNA in mouse small intestinal crypt epithelium, *J. Cell Biol.* **108:**1687–1695.
74. Selsted, M. E., Szklarek, D., Ganz, T., and Lehrer, R. I., 1985, Activity of rabbit leukocyte peptides against *Candida albicans*, *Infect. Immun.* **49:**202–206.
75. Krause, W., Matheis, H., and Wulf, K., 1969, Fungaemia and funguria after oral administration of *Candida albicans*, *Lancet* **1:**598–599.
76. Sass, W., Dreyer, H. P., Bockeler, W., Hamelmann, H., and Seifert, J., 1987, Prinzipien der partikelresorption im magen-darm-trakt, *Z. Gastroenterol.* **25:**306–315.
77. Baine, W. B., Koenig, M. G., and Goodman, J. S., 1974, Clearance of *Candida albicans* from the bloodstream of rabbits, *Infect. Immun.* **10:**1420–1425.
78. Bird, D. C., and Sheagren, J. N., 1970, Evaluation of reticuloendothelial system phagocytic activity during systemic *Candida albicans* infection in mice, *Proc. Soc. Exp. Biol. Med.* **133:**34–37.
79. Cho, Y. S., and Choi, H. Y., 1979, Opportunistic fungal infection among cancer patients: a ten year autopsy study, *Am. J. Clin. Pathol.* **72:**617–620.
80. Bach, M. C., Sahyoun, A., Adler, J. L., Schlesinger, R. M., Breman, J., Madras, P., P'eng, F. K., and Monaco, A. P., 1973, High incidence of fungus infections in renal transplantation patients treated with antilymphocyte and conventional immunosuppression, *Transplant. Proc.* **5:**549–553.

81. Louria, D. B., Brayton, R. G., and Finkel, G., 1963, Studies on the pathogenesis of experimental *Candida albicans* infections in mice, *Sabouraudia* **2**:271–283.
82. Ruthe, R. C., Andersen, B. R., Cunningham, B. L., and Epstein, R. B., 1978, Efficacy of granulocyte transfusions in control of systemic candidiasis in leukopenic host, *Blood* **52**: 493–498.
83. Hurtrel, B., Lagrange, P. H., and Michel, J.-C., 1980, Systemic candidiasis in mice. II. Main role of polymorphonuclear leukocytes in resistance to infection, *Ann. D'Immunol.* **131**:105–118.
84. Cutler, J. E., and Poor, A. H., 1981, Effect of mouse phagocytes on *Candida albicans* in in vivo chambers, *Infect. Immun.* **31**:1110–1116.
85. Lehrer, R. I., and Cline, M. J., 1969, Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to *Candida* infection, *J. Clin. Invest.* **48**:1478–1488.
86. Cech, P., Papathanassiou, A., Boreux, G., Roth, P., and Miescher, P. A., 1979, Hereditary myeloperoxidase deficiency, *Blood* **53**:403–411.
87. Cech, P., Stalder, H., Widmann, J.-J., Rohner, A., and Miescher, P. A., 1979, Leukocyte myeloperoxidase deficiency and diabetes mellitus associated with *Candida albicans* liver abscess, *Am. J. Med.* **66**:149–153.
88. Moosman, K., and Bojanovsky, A., 1975, Rezidivierende candidosis bei myeloperoxydase mangel, *Monatschr. Kinderheilkd.* **123**:408–409.
89. Parry, M. F., Root, R. K., Metcalf, J. A., Delaney, K. K., Kaplow, L. S., and Richar, W. J., 1981, Myeloperoxidase deficiency. Prevalence and clinical significance, *Ann. Intern. Med.* **95**: 293–301.
90. Catovsky, D., Galton, D. A., Robinson, J., 1972, Myeloperoxidase-deficient neutrophils in acute myeloid leukaemia, *Scand. J. Haematol.* **9**:142–148.
91. Bendix-Hansen, K., and Nielsen, H. K., 1983, Myeloperoxidase-deficient polymorphonuclear leucocytes (II). Longitudinal study in acute myeloid leukaemia, untreated, in remission and in relapse, *Scand. J. Haematol.* **31**:5–8.
92. Bendix-Hansen, K., Kerndrup, G., and Pedersen, B., 1986, Myeloperoxidase-deficient polymorphonuclear leucocytes (VI): Relation to cytogenetic abnormalities in primary myelodysplastic syndromes, *Scand. J. Haematol.* **36**:3–7.
93. Lehrer, R. I., Goldberg, L. S., Apple, M. A., and Rosenthal, N. P., 1972, Refractory megaloblastic anemia with myeloperoxidase-deficient neutrophils, *Ann. Intern. Med.* **76**: 447–453.
94. Lehrer, R. I., 1972, Functional aspects of a second mechanism of candidacidal activity by human neutrophils, *J. Clin. Invest.* **51**:2566–2572.
95. Lehrer, R. I., Hanifin, J., and Cline, M. J., 1969, Defective bactericidal activity in myeloperoxidase-deficient human neutrophils, *Nature (Lond.)* **223**:78–79.
96. Lehrer, R. I., 1975, The fungicidal mechanisms of human monocytes. I. Evidence for myeloperoxidase-linked and myeloperoxidase-independent candidacidal mechanisms, *J. Clin. Invest.* **55**:338–346.
97. Diamond, R. D., Krzesicki, R., and Jao, W., 1978, Damage to pseudohyphal forms of *Candida albicans* by neutrophils in the absence of serum *in vitro*, *J. Clin. Invest.* **61**:349–359.
98. Rosner, F., Valmont, I., Kozinn, P. J., and Caroline, L., 1970, Leukocyte function in patients with leukemia, *Cancer* **25**:835–842.
99. Lehrer, R. I., and Cline, M. J., 1971, Leukocyte candidacidal activity and resistance to systemic candidiasis in patients with cancer, *Cancer* **27**:1211–1217.
100. Goldman, J. M., and Th'ng, K. H., 1973, Phagocytic function of leukocytes from patients with acute myeloid and chronic granulocytic leukaemia, *Br. J. Haematol.* **25**:299–308.
101. Bjerknes, R., Vindenes, H., and Laerum, O. D., 1990, Altered neutrophil functions in patients with large burns, *Blood Cells* **16**:127–141.
102. El-Maalem, H., and Fletcher, J., 1976, Defective neutrophil function in chronic granulocytic leukaemia, *Br. J. Haematol.* **66**:95–103.

103. Child, J. A., Martin, S., Cawley, J. C., and Ghoniem, A. T. M., 1978, Defective microbicidal function of neutrophils in haematological malignancies and lymphomas: correction by levamisole in vitro, *Biomedicine* **29:**159–161.
104. Uberti-Foppa, C., Franzetti, F., Hegri, C., Novati, R., Cinque, P., Privitera, G., Parente, F., Crocchiolo, P., and Lazzarin, A., 1987, Role of PMN's candidacidal activity in the pathogenesis of AIDS-related systemic candidiasis, *Bull. Ist. Sieroter. Milan* **66:**471–478.
105. Xanthou, M., Valassi-Adam, E., Kintsonidou, E., and Matsaniotis, N., 1975, Phagocytosis and killing ability of *Candida albicans* by blood leucocytes of healthy term and preterm babies, *Arch. Dis. Child* **50:**72–75.
106. Quie, P. G., and Chilgren, R. A., 1971, Acute disseminated and chronic mucocutaneous candidiasis, *Semin. Hematol.* **8:**227–242.
107. Oseas, R., and Lehrer, R. I., 1978, A micromethod for measuring neutrophil candidacidal activity in neonates, *Pediatr. Res.* **12:**828–829.
108. Tuck, R., Burke, V., Gracey, M., Malajczuk, A., and Sunoto, 1979, Defective *Candida* killing in childhood malnutrition, *Arch. Dis. Child.* **54:**445–447.
109. Boyne, R., Arthur, J. R., and Wilson, A. B., 1986, An in vivo and in vitro study of selenium deficiency and infection in rats, *J. Comp. Pathol.* **96:**379–386.
110. Kernbaum, S., and Vilde, J.-L., 1974, Etude du pouvoir candidacide des polynucleaires neutrophiles humains, *Pathol. Biol. (Paris)* **22:**61–66.
111. El Falaky, I. H., and Shoukry, H. M., 1977, Phagocytosis and intracellular killing of *Candida albicans* by polymorphonuclear leucocytes in thalassaemia, *J. Egyptian Public Health Assoc.* **52:**317–329.
112. Drutz, D. J., and Lehrer, R. I., 1978, Development of amphotericin B-resistant *Candida tropicalis* in a patient with defective leukocyte function, *Am. J. Med. Sci.* **276:**77–92.
113. Edwards, J. E., Jr., Rotrosen, D., Fontaine, J. W., Haudenschild, C. C., and Diamond, R. D., 1987, Neutrophil-mediated protection of cultured human vascular endothelial cells from damage by growing *Candida albicans* hyphae, *Blood* **69:**1450–1457.
114. Retrosen, 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:**1264–1274.
115. Davies, R. R., and Denning, T. J. V., 1972, *Candida albicans* and the fungicidal activity of the blood, *Sabouraudia* **10:**301–312.
116. Lehrer, R. I., and Cline, M. J., 1969, Interaction of *Candida albicans* with human leukocytes and serum, *J. Bacteriol.* **98:**996–1004.
117. Edwards, J. E., Jr., Lehrer, R. I., Stiehm, E. R., Fischer, T. J., and Young, L. S., 1978, Severe candidal infections. Clinical perspective, immune defense mechanisms, and current concepts of therapy, *Ann. Intern. Med.* **89:**91–106.
118. Ray, T. L., and Wuepper, K. D., 1976, Activation of the alternative (properdin) pathway of complement by *Candida albicans* and related species, *J. Invest. Derm.* **67:**700–703.
119. Denning, T. J. V., and Davies, R. R., 1973, *Candida albicans* and the chemotaxis of polymorphonuclear neutrophils, *Sabouraudia* **11:**210–221.
120. Weeks, B. A., Escobar, M. R., Hamilton, P. B., and Fueston, V. M., 1976, Chemotaxis of polymorphonuclear neutrophilic leukocytes by mannan-enriched preparations of *Candida albicans*, *Adv. Exp. Med. Biol.* **73:**161–169.
121. Ray, T. L., Hanson, A., Ray, L. F., and Wuepper, K. D., 1979, Purification of a mannan from *Candida albicans* which activates serum complement, *J. Invest. Dermatol.* **73:**269–274.
122. Cutler, J. E., 1977, Chemotactic factor produced by *Candida albicans*, *Infect. Immun.* **18:** 568–573.
123. Morelli, R., and Rosenberg, L. T., 1971, The role of complement in the phagocytosis of *Candida albicans* by mouse peripheral blood leukocytes, *J. Immunol.* **107:**476–480.
124. Yamamura, M., and Valdimarsson, H., 1977, Participation of C3 in intracellular killing of *Candida albicans*, *Scand. J. Immunol.* **6:**591–594.

125. Ferrante, A., and Thong, Y. H., 1979, Requirement of heat-labile opsonins for maximal phagocytosis of *Candida albicans*, *Sabouraudia* **17**:293–297.
126. Kernbaum, S., 1975, Pouvoirs phagocytaire et fongicide envers *Candida albicans* des polynucleaires neutrophiles humains en presence de serum depourvu de C3 et C4, *Ann. Microbiol. (Inst. Pasteur).* **126A**:75–81.
127. Morrison, R. P., and Cutler, J. E., 1981, *In vitro* studies of the interaction of murine phagocytic cells with *Candida albicans*, *J. Reticuloendothel. Soc.* **29**:23–24.
128. Sinski, J. T., Reed, G. L., Kelley, L. M., and Le Vebvre, R., 1973, Macrophage migration technique using coccidioidin, *Infect. Immun.* **7**:226–230.
129. Solomkin, J. S., Mills, E. L., Giebink, G. S., Nelson, R. D., Simmons, R. L., and Quie, P. G., 1978, Phagocytosis of *Candida albicans* by human leukocytes: opsonic requirements, *J. Infect. Dis.* **137**:30–37.
130. Leijh, P. C., van den Barselaar, M. T., and van Furth, R., 1977, Kinetics of phagocytosis and intracellular killing of *Candida albicans* by human granulocytes and monocytes, *Infect. Immun.* **17**:313–318.
131. Ginsburg, I., Sela, M. N., Morag, A., Ravid, Z., Duchan, Z., Ferne, M., Rabinowitz-Bergner, S., Thomas, P. P., Davies, P., Niccols, J., Humes, J., and Bonney, R., 1981, Role of leukocyte factors and cationic polyelectrolytes in phagocytosis of group A streptococci and *Candida albicans* by neutrophils, macrophages, fibroblasts and epithelial cells: modulation by anionic polyelectrolytes in relation to pathogenesis of chronic inflammation, *Inflammation* **5**:289–312.
132. Pereira, H. A., and Hosking, C. S., 1984, The role of complement and antibody in opsonization and intracellular killing of *Candida albicans*, *Clin. Exp. Immunol.* **57**:307–314.
133. Sandhu, D. K., Sandhu, R. S., Damodaran, V. N., and Randhawa, H. S., 1970, Effect of cortisone on bronchopulmonary aspergillosis in mice exposed to spores of various *Aspergillus* species, *Sabouraudia* **8**:32–38.
134. Hurley, D. L., Balow, J. E., and Fauci, A. S., 1975, Experimental disseminated candidiasis. II. Administration of glucocorticosteroids, susceptibility to infection and immunity, *J. Infect. Dis.* **132**:393–398.
135. Laforce, F. M., Mills, D. M., Iverson, K., Cousins, R., and Everett, E. D., 1975, Inhibition of leukocyte candidacidal activity by serum from patients with disseminated candidiasis, *J. Lab. Clin. Med.* **86**:657–666.
136. Walker, S. M., and Urbaniak, S. J., 1980, A serum-dependent defect of neutrophil function in chronic mucocutaneous candidiasis, *J. Clin. Pathol.* **33**:370–372.
137. Fleischmann, J., Selsted, M. E., and Lehrer, R. I., 1985, Opsonic activity of MCP-1 and MCP-2, cationic peptides from rabbit alveolar macrophages, *Diagn. Microbiol. Infect. Dis.* **3**: 233–242.
138. Hector, R. F., Domer, J. E., and Carrow, E. W., 1982, Immune responses to *Candida albicans* in genetically-distinct mice, *Infect. Immun.* **38**:1020–1028.
139. Lyon, F. L., Hector, R. F., and Domer, J. E., 1986, Innate and acquired immune responses against *Candida albicans* in congenic B10.D2 mice with deficiency of the C5 complement component, *J. Med. Vet. Mycol.* **24**:359–367.
140. Gelfand, J. A., Hurley, D. L., Fauci, A. S., and Frank, M. M., 1978, Role of complement in host defense against experimental disseminated candidiasis, *J. Infect. Dis.* **138**:9–16.
141. Lehrer, R. I., 1970, Measurement of candidacidal activity of specific leukocyte types in mixed cell populations. I. Normal, myeloperoxidase-deficient, and chronic granulomatous disease neutrophils, *Infect. Immun.* **2**:42–47.
142. Husseini, R. H., Hoadley, M. E., Hutchinson, J. J. P., Penn, C. W., and Smith, H., 1985, Intracellular killing of *Candida albicans* by human polymorphonuclear leucocytes: comparison of three methods of assessment, *J. Immunol. Methods* **81**:215–221.
143. Lehrer, R. I., 1971, Inhibition by sulfonamides of the candidacidal activity of human neutrophils, *J. Clin. Invest.* **50**:2498–2505.

144. Schmid, L., and Brune, K., 1974, Assessment of phagocytic and antimicrobial activity of human granulocytes, *Infect. Immun.* **10:**1120–1126.
145. Vecchiarelli, A., Bistoni, F., Cenci, E., Perito, S., and Cassone, A., 1985, In-vitro killing of *Candida* species by murine immunoeffectors and its relationship to the experimental pathogenicity, *Sabouraudia* **23:**377–387.
146. Scherwitz, C., and Martin, R., 1979, The phagocytosis of *Candida albicans* blastospores and germ tubes by polymorphonuclear leukocytes, *Dermatologia* **159:**12–23.
147. Schuit, K. E., 1979, Phagocytosis and intracellular killing of pathogenic yeast by human monocytes and neutrophils, *Infect. Immun.* **24:**932–938.
148. Cockayne, A., and Odds, F. C., 1984, Interactions of *Candida albicans* yeast cells, germ tubes, and hyphae with human polymorphonuclear leucocytes in vitro, *J. Gen. Microbiol.* **130:** 465–471.
149. Brummer, E., McEwen, J. G., and Stevens, D. A., 1986, Fungicidal activity of murine inflammatory polymorphonuclear neutrophils: comparison with murine peripheral blood PMN, *Clin. Exp. Immunol.* **66:**681–690.
150. Kernbaum, S., 1975, Pouvoir candidacide des polynucleaires neutrophiles humains et chimiotherapie antibacterienne, *Pathol. Biol.* **22:**789–794.
151. Ferrari, F. A., Pagani, A., Marconi, M., Stefanoni, R., and Siccardi, A. G., 1980, Inhibition of candidacidal activity of human neutrophil leukocytes by aminoglucoside antibiotics, *Antimicrob. Agents Chemother.* **17:**87–88.
152. Roilides E., Walsh, T. J., Rubin, M., Venzon, D., and Pizzo, P. A., 1990, Effects of antifungal agents on the function of human neutrophils in vitro, *Antimicrob. Agents Chemother.* **34:** 196–201.
153. Meshulam, T., Diamond, R. D., Lyman, C. A., Wysong, D. R., and Melnick, D. A., 1988, Temporal association of calcium mobilization, inositol triphosphate generation, and superoxide anion release by human neutrophils activated by serum opsonized and nonopsonized particulate stimuli, *Biochem. Biophys. Res. Commun.* **150:**532–539.
154. Wilson, R. M., and Reeves, W. G., 1986, Neutrophil phagocytosis and killing in insulin-dependent diabetes, *Clin. Exp. Immunol.* **63:**478–484.
155. Chan, C. K., and Balish, E., 1978, Inhibition of granulocyte phagocytosis of *Candida albicans* by amphotericin B, *Can. J. Microbiol.* **24:**363–364.
156. Belcher, R. W., Carney, J. F., and Monahan, F. G., 1973, An electron microscopic study of phagocytosis of *Candida albicans* by polymorphonuclear leukocytes, *Lab. Invest.* **29:** 620–627.
157. Richardson, M., and Smith, H., 1983, Ultrastructural features of phagocytosis and intracellular killing of *Candida albicans* by mouse polymorphonuclear phagocyte monolayers, *Mycopathologia* **83:**97–102.
158. Cech, P., and Lehrer, R. I., 1984, Heterogeneity of human neutrophil phagolysosomes: functional consequences for candidacidal activity, *Blood* **64:**147–151.
159. Levitz, S. M., DiBenedetto, D. J., and Diamond, R. D., 1987, A rapid fluorescent assay to distinguish attached from phagocytized yeast particles, *J. Immunol. Methods* **101:**37–42.
160. Klebanoff, S. J., 1980, Oxygen metabolism and the toxic properties of phagocytes, *Ann. Intern. Med.* **93:**480–489.
161. Weiss, S. J., Test, S. T., Eckmann, C. M., Roos, D., and Regiani, S., 1986, Brominating oxidants generated by human eosinophils, *Science* **234:**200–203.
162. Lehrer, R. I., 1971, Measurement of candidacidal activity of specific leukocyte types in mixed cell populations: II. Normal and chronic granulomatous disease eosinophils, *Infect. Immun.* **3:**800–802.
163. Foote, C. S., Goyne, T. E., and Lehrer, R. I., 1983, Assessment of chlorination by human neutrophils, *Nature* **301:**715–716.
164. Weiss, S. J., Klein, R., Slivka, A., and Wei, M., 1982, Chlorination of taurine by human neutrophils: evidence for hypochlorous acid generation, *J. Clin. Invest.* **70:**598–607.

165. Lehrer, R. I., 1969, Antifungal effects of peroxidase systems, *J. Bacteriol.* **99:**361–365.
166. Lehrer, R. I., and Jan, R. G., 1970, Interaction of *Aspergillus fumigatus* spores with human leukocytes and serum, *Infect. Immun.* **1:**345–350.
167. Diamond, R. D., Clark, R. A., and Haudenschild, C. C., 1980, Damage to *Candida albicans* hyphae and pseudohyphae by the myeloperoxidase system and oxidative products of neutrophil metabolism *in vitro*, *J. Clin. Invest.* **66:**908–917.
168. Wagner, D. K., Collins-Lech, C., and Sohnle, P. G., 1986, Inhibition of neutrophil killing of *Candida albicans* pseudohyphae by substances which quench hypochlorous acid and chloramines, *Infect. Immun.* **51:**731–735.
169. Beilke, M. A., Collins-Lech, C., and Sohnle, P. G., 1989, Candidacidal activity of the neutrophil myeloperoxidase system can be protected from excess hydrogen peroxide by the presence of ammonium ion, *Blood* **73:**1045–1049.
170. Kolotila, M. P., and Diamond, R. D., 1990, Effects of neutrophils and *in vitro* oxidants on survival and phenotypic switching of *Candida albicans* W0-1, *Infect. Immun.* **58:**1174–1179.
171. Babior, B. M., 1988, The respiratory burst oxidase, in, Phagocytic defects II (Curnutte, J. J., ed). *Hematol. Oncol Clin. North Am.* **2:**201–212.
172. Clark, R. A., 1990, Perspective: The human neutrophil respiratory burst oxidase, *J. Infect. Dis.* **161:**1140–1147.
173. Cohen, M. S., Isturiz, R. E., Malech, H. L., Root, R. K., Wilfert, C. M., Gutman, L., and Buckley, R. H., 1981, Fungal infection in chronic granulomatous disease—the importance of the phagocyte in defense against fungi, *Am. J. Med.* **71:**59–66.
174. Lehrer, R. I., Ladra, K. M., and Hake, R. B., 1975, Nonoxidative fungicidal mechanisms of mammalian granulocytes: demonstration of components with candidacidal activity in human, rabbit, and guinea pig leukocytes, *Infect. Immun.* **11:**1226–1234.
175. Levitz, S. M., and Diamond, R. D., 1984, Killing of *Aspergillus fumigatus* spores and *Candida albicans* yeast phase by the iron-hydrogen peroxide-iodide cytotoxic system: comparison with the myeloperoxidase-hydrogen peroxide-halide system, *Infect. Immun.* **43:**1100–1102.
176. Lehrer, R. I., Szklarek, D., Ganz, T., and Selsted, M. E., 1985, Correlation of binding of rabbit granulocyte peptides to *Candida albicans* with candidacidal activity, *Infect. Immun.* **49:** 207–211.
177. Lehrer, R. I., Szklarek, D., Ganz, T., and Selsted, M. E., 1986, Synergistic activity of rabbit granulocyte peptides against *Candida albicans*, *Infect. Immun.* **52:**902–904.
178. Diamond, R. D., and Krzesicki, R., 1978, Mechanism of attachment of neutrophils to *Candida albicans* pseudohyphae in the absence of serum, and of subsequent damage to pseudohyphae by microbicidal processes of neutrophils *in vitro*, *J. Clin. Invest.* **61:**360–369.
179. Levitz, S. M., Lyman, C. A., Murata, T., Sullivan, J. A., Mandell, G. L., and Diamond, R. D., 1987, Cytosolic calcium changes in individual neutrophils stimulated by opsonized and unopsonized *Candida albicans* hyphae, *Infect. Immun.* **55:**2783–2788.
180. Lyman, C. A., Simons, E. R., Melnick, D. A., and Diamond, R. D., 1987, Unopsonized *Candida albicans* hyphae stimulate a neutrophil respiratory burst and a cytosolic calcium flux without membrane depolarization, *J. Infect. Dis.* **156:**770–776.
181. Lyman, C. A., Simons, E. R., Melnick, D. A., and Diamond, R.D ., 1988, Induction of signal transduction in human neutrophils by *Candida albicans* hyphae: the role of pertussis toxin-sensitive guanosine triphosphate-binding proteins, *J. Infect. Dis.* **158:**1056–1064.
182. Kolotila, M. P., and Diamond, R. D., 1988, Stimulation of neutrophil actin polymerization and degranulation by opsonized and unopsonized *Candida albicans* hyphae and zymosan, *Infect. Immun.* **56:**2016–2022.
183. Wysong, D. R., Lyman, C. A., and Diamond, R. D., 1989, Independence of neutrophil respiratory burst oxidant generation from the early cytosolic calcium response after stimulation with unopsonized *Candida albicans* hyphae, *Infect. Immun.* **57:**1499–1505.
184. Diamond, R. D., and Noble, L., 1990, Patterns of guanine nucleotide exchange reflecting

disparate neutrophil activation pathways by opsonized and unopsonized *Candida albicans* hyphae, *J. Infect. Dis.* **162:**262–265.
185. Sawyer, D. W., Donowitz, G. R., and Mandell, G. L., 1989, Polymorphonuclear neutrophils: an effective antimicrobial force, *Rev. Infect. Dis.* **11:**S1532–S1544.
186. Lehrer, R. I., and Ganz, T., 1990, Antimicrobial polypeptides of human neutrophils, *Blood* **76:**2169–2181.
187. Zeya, H. I., and Spitznagel, J. K., 1966, Cationic proteins of polymorphonuclear leukocyte lysosomes. I. Resolution of antibacterial and enzymatic activities, *J. Bacteriol.* **91:**750–754.
188. Lehrer, R. I., Ganz, T., and Selsted, M. E., 1990, Defensins: natural peptide antibiotics from neutrophils, *ASM News* **56:**315–318.
189. Ganz, T., Selsted, M. E., and Lehrer, R. I., 1990, Defensins, *Eur. J. Haematol.* **44:**1–8.
190. Lehrer, R. I., Ganz, T., Szklarek, D., and Selsted, M. E., 1988, Modulation of the *in vitro* candidacidal activity of human neutrophil defensins by target cell metabolism and divalent cations, *J. Clin. Invest.* **81:**1829–1835.
191. Rice, W. G., Ganz, T., Kinkade, J. M., Jr., Selsted, M. E., Lehrer, R. I., and Parmley, R. T., 1987, Defensin-rich dense granules of human neutrophils, *Blood* **70:**757–765.
192. Wilde, C. G., Griffith, J. E., Marra, M. N., Snable, J. L., and Scott, R. W., 1989, Purification and characterization of human neutrophil peptide 4, a novel member of the defensin family, *J. Biol. Chem.* **264:**11200–11203.
193. Selsted, M. E., Brown, D. M., DeLange, R. J., Harwig, S. S., and Lehrer, R. I., 1985, Primary structures of six antimicrobial peptides of rabbit peritoneal neutrophils, *J. Biol. Chem.* **260:** 4579–4584.
194. Eisenhauer, P. B., Harwig, S. S. L., Szklarek, D., Ganz, T., Selsted, M. E., and Lehrer, R. I., 1989, Purification and antimicrobial properties of three defensins from rat neutrophils, *Infect. Immun.* **57:**2021–2027.
195. Eisenhauer, P., Harwig, S. S. S. L., Szklarek, D., Ganz, T., and Lehrer, R. I., 1990, Polymorphic expression of defensins in neutrophils from outbred rats, *Infect. Immun.* **58:** 3899–3902.
196. Kagan, B. L., Selsted, M. E., Ganz, T., and Lehrer, R. I., 1990, Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes, *Proc. Natl. Acad. Sci. USA* **87:**210–214.
197. Odeberg, H., and Olsson, I., 1975, Antibacterial activity of cationic proteins from human granulocytes, *J. Clin. Invest.* **56:**1118–1124.
198. Odeberg, H., and Olsson, I., 1976, Microbicidal mechanisms of human granulocytes: synergistic effects of granulocyte elastase and myeloperoxidase or chymotrypsin-like cationic protein, *Infect. Immun.* **14:**1276–1283.
199. Gabay, J. E., Scott, R. W., Campanelli, D., Griffith, J., Wilde, C., 1989, Antibiotic proteins of human polymorphonuclear leukocytes, *Proc. Natl. Acad. Sci. USA* **86:**5610–5614.
200. Campanelli, D., Detmers, P. A., Nathan, C. F., Gabay, J. E., 1990, Azurocidin and a homologous serine protease from neutrophils. Differential antimicrobial and proteolytic properties, *J. Clin. Invest.* **85;**904–915.
201. Wilde, C. G., Snable, J. L., Griffith, J. E., Scott, R. W., 1990, Characterization of two azurophil granule proteases with active-site homology to neutrophil elastase, *J. Biol. Chem.* **265:**2038–2041.
202. Shafer, W. M., Pohl, J., Onunka, V. C., Bangalore, N., and Travis, J., 1990, Human lysosomal cathepsin G and granzyme B share a functionally conserved broad spectrum antibacterial peptide, *J. Biol. Chem.* **266:**112–116.
203. Arnold, R. R., Brewer, M., and Gauthier, J. J., 1980, Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms, *Infect. Immun.* **28:**893–898.
204. Collins, M. S., and Pappagianis, D., 1973, Effects of lysozyme and chitinase on the spherules of *Coccidioides immitis in vitro*, *Infect. Immun.* **7:**817–822.

205. Gadebusch, H. H., and Johnson, A. G., 1966, Natural host resistance to infection with *Cryptococcus neoformans*. IV. The effect of some cationic proteins on the experimental disease, *J. Infect. Dis.* **116:**551–565.
206. Rausch, P. G., and Moore, T. G., 1975, Granule enzymes of polymorphonuclear neutrophils: a phylogenetic comparison, *Blood* **46:**913–919.
207. Marquis, G., Montplaisir, S., Garzon, S., Strykowski, H., and Auger, P., 1982, Fungitoxicity of muraminidase: ultrastructural damage to *Candida albicans*, *Lab. Invest.* **46:**627–636.
208. Kamaya, T., 1970, Lytic action of lysozyme on *Candida albicans*, *Mycopathol. Mycol. Appl.* **42:** 197–207.
209. Ray, T. L., and Wuepper, K. D., 1976, Experimental cutaneous candidiasis in rodents, *J. Invest. Derm.* **66:**29–33.
210. Ray, T. L., and Wuepper, K. D., 1978, Experimental cutaneous candidiasis in rodents II. Role of the stratum corneum barrier and serum complement as a mediator of a protective inflammatory response, *Arch. Dermatol.* **114:**539–543.
211. Louria, D. B., and Brayton, R. G., 1964, Behavior of *Candida* cells within leukocytes, *Proc. Soc. Exp. Biol. Med.* **115:**93–98.
212. Diamond, R. D., Oppenheim, F., Nakagawa, Y., Krzesicki, R., and Haudenschild, C. C., 1980, Properties of a product of *Candida albicans* hyphae and pseudohyphae that inhibits contact between the fungi and human neutrophils *in vitro*, *J. Immunol.* **125:**2797–2804.
213. Smail, E. H., Melnick, D. A., Ruggeri, R., and Diamond, R. D., 1988, A novel natural inhibitor from *Candida albicans* hyphae causing dissociation of the neutrophil respiratory burst response to chemotactic peptides from other post-activating events, *J. Immunol.* **140:** 3893–3899.
214. Smail, E. H., Kolotila, M. P., Ruggeri, R., and Diamond, R. D., 1989, Natural inhibitor from *Candida albicans* blocks release of azurophil and specific granule contents by chemotactic peptide-stimulated human neutrophils, *Infect. Immun.* **57:**689–692.
215. Hilger, A. E., and Danley, D. L., 1980, Alteration of polymorphonuclear leukocyte activity by viable *Candida albicans*, *Infect. Immun.* **27:**714–720.
216. Danley, D. L., Hilger, A. E., Winkel, C. A., 1983, Generation of hydrogen peroxide by *Candida albicans* and influence on murine polymorphonuclear leukocyte activity, *Infect. Immun.* **40:**97–102.
217. Heidenreich, F., and Dierich, M. P., 1985, *Candida albicans* and *Candida stellatoidea* in contrast to other *Candida* species bind iC3b and C3d but not C3b, *Infect. Immun.* **50:**598–600.
218. Edwards, J. E., Jr., Gaither, T. A., O'Shea, J. J., Rotrosen, D., Lawley, T. J., Wright, S. A., Frank, M. M., and Green, I., 1986, Expression of specific binding sites on *Candida* with functional and antigenic characteristics of human complement receptors, *J. Immunol.* **137:** 3577–3583.
219. Calderone, R. A., Linehan, L., Wadsworth, E., and Sandberg, A. L., 1988, Identification of C3d receptors on *Candida albicans*, *Infect. Immun.* **56:**252–258.
220. 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.
221. 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:** 616–622.
222. Hostetter, M. K., 1990, Handicaps to host defense. Effects of hyperglycemia on C3 and *Candida albicans*, *Diabetes* **39:**271–275.
223. Hostetter, M. K., Lorenz, J. S., Preus, L., 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.

224. Slutsky, B., Buffo, J., and Soll, D. R., 1985, High-frequency switching of colony morphology in *Candida albicans*, *Science* **230:**666–669.
225. Soll, D. R., Slutsky, B., Mackenzie, S., Langtimm, C., and Staebell, M., 1987, Two newly discovered switching systems in *Candida albicans* and their possible role in oral candidiasis, in: *Oral mucosal diseases: biology, etiology, and therapy*, (I. C. Mackenzie, C. A. Squier, and E. Dabelsteen, eds.), Laegeforeningen Forlag, Copenhagen, pp. 52–59.
226. Ishikawa, T., Dalton, A. C., and Arbesman, C. E., 1972, Phagocytosis of *Candida albicans* by eosinophilic leukocytes, *J. Allergy Clin. Immunol.* **49:**311–315.
227. Ishikawa, T., Yu, M. C., and Arbesman, C. E., 1972, Electron microscopic demonstration of phagocytosis of *Candida albicans* by human eosinophilic leukocytes, *J. Allergy Clin. Immunol.* **50:**183–187.
228. Lehrer, R. I., 1981, Ingestion and destruction of *Candida albicans*, in: *Methods for Studying Mononuclear Phagocytes*, (D. A. Adams, H. Koren, and P. Edelson, eds.), Academic, New York, pp. 693–708.
229. Estevez, M. E., Sen, L., Vadez, R., and Balina, L. M., 1979, Defective blood mononuclear phagocyte function in patients with leprosy, *Int. J. Leprosy* **47:**575–579.
230. Estevez, M. E., Ballart, I. J., Diez, R. A., Planes, N., Scaglione, C., and Sen, L., 1986, Early defect of phagocytic cell function in subjects at risk for acquired immunodeficiency syndrome, *Scand. J. Immunol.* **24:**215–221.
231. Cohen, A. B., and Cline, M. J., 1971, The human alveolar macrophage: isolation, cultivation *in vitro*, and studies of morphologic and functional characteristics, *J. Clin. Invest.* **50:**1390–1398.
232. Kemmerich, B., Rossing, T. H., and Pennington, J. E., 1987, Comparative oxidative microbicidal activity of human blood monocytes and alveolar macrophages and activation by recombinant gamma interferon, *Am. Rev. Respir. Dis.* **136:**266–270.
233. Territo, M. C., and Golde, D. W., 1979, The function of human alveolar macrophages, *J. Reticuloendothel. Soc.* **25:**111–120.
234. Winston, D. J., Territo, M. C., Ho, W. G., Miller, M. J., Gale, R. P., and Golde, D. W., 1982, Alveolar macrophage dysfunction in human bone marrow transplant recipients, *Am. J. Med.* **73:**859–866.
235. Golde, D. W., Territo, M., Finley, T. N., and Cline, M. J., 1976, Defective lung macrophages in pulmonary alveolar proteinasis, *Ann. Intern. Med.* **85:**304–309.
236. Robinson, J. E., Harvey, B. A. M., and Soothill, J. F., 1978, Phagocytosis and killing of bacteria and yeast by human milk cells after opsonisation in aqueous phase of milk, *Br. Med. J.* **1:**1443–1445.
237. Taschdjian, C. L., Toni, E. F., Hsu, K. C., Seelig, M. S., Cuesta, M. B., and Kozinn, P. J., 1971, Immunofluorescence studies of *Candida* in human reticuloendothelial phagocytes: implications for immunogenesis and pathogenesis of systemic candidiasis, *Am. J. Clin. Pathol.* **56:** 50–58.
238. Odegaard, A., Viken, K. E., and Lamvik, J., 1974, Structural and functional properties of blood monocytes cultured *in vitro*, *Acta Pathol. Microbiol. Scand.* Sect. G. **82:**223–234.
239. Lehrer, R. I., 1970, The fungicidal activity of human monocytes: a myeloperoxidase-linked mechanism, *Clin. Res.* **18:**408–413.
240. Sasada, M., Kubo, A., Nishimura, T., Kakita, T., Moriguchi, T., Yamamoto, K., and Uchino, H., 1987, Candidacidal activity of monocyte-derived human macrophages: relationship between *Candida* killing and oxygen radical generation by human macrophages, *J. Leukoc. Biol.* **41:**289–294.
241. Bourne, H. R., Lehrer, R. I., Cline, M. J., and Melmon, K. L., 1971, Cyclic 3′,5′-adenosine monophosphate in the human leukocyte: synthesis, degradation, and effects on neutrophil candidacidal activity, *J. Clin. Invest.* **50:**920–929.
242. Odegaard, A., and Lamvik, J., 1976, The effect of phenylbutazone and chloramphenicol on

phagocytosis of radiolabelled *Candida albicans* by human monocytes cultured *in vitro*, *Acta Pathol. Microbiol. Scand.* Sect. C **84:**37–44.

243. Louria, D. B., Fallon, N., and Browne, H. G., 1960, The influence of cortisone on experimental fungus infections in mice, *J. Clin. Invest.* **39:**1435–1449.
244. Diamond, R. D., 1981, Mechanisms of host resistance to *Candida albicans*. In *Microbiology*, 1981, American Society for Microbiology, Washington, D. C., pp. 200–204.
245. Rinehart, J. J., Sagone, A. L., Balcerzak, S. P., Ackerman, G. A., and LoBuglio, A. F., 1975, Effects of corticosteroid therapy on human monocyte function, *N. Engl. J. Med.* **292:**236–241.
246. Hammerstrom, J., and Unsgaard, G., 1979, *In vitro* influence of endotoxin on human mononuclear phagocyte structure and function, *Acta Pathol. Microbiol. Scand.* **Sect. C 87:** 381–389.
247. Ganz, T., Selsted, M. E., Szklarek, D., Harwig, S. S. L., Daher, K., Bainton, D. F., and Lehrer, R. I., 1985, Defensins: natural peptide antibiotics of human neutrophils, *J. Clin. Invest.* **76:** 1427–1435.
248. Campbell, E. J., Silverman, E. K., and Campbell, M. A., 1989, Elastase and cathepsin G of human monocytes. Quantification of cellular content, release in response to stimuli and heterogeneity in elastase-mediated proteolytic activity, *J. Immunol.* **143:**2961–2968.
249. Hurtrel, B., and Lagrange, P. H., 1981, Comparative effects of carrageenan on systemic candidiasis and listeriosis in mice, *Clin. Exp. Immunol.* **44:**355–358.
250. Lee, K. W., and Balish, E., 1983, Systemic candidosis in silica-treated athymic and euthymic mice, *Infect. Immun.* **41:**902–907.
251. Sinha, B. K., Monga, D. P., and Prasad, S., 1987, Studies of the role of macrophages in experimental candidosis in mice, *Mykosen* **30:**105–112.
252. Baghian, A., and Lee, K. W., 1988, Role of activated macrophages in resistance to systemic candidosis, *J. Leukoc. Biol.* **44:**166–171.
253. Cheers, C., and Waller, R., 1975, Activated macrophages in congenitally athymic "nude" mice and in lethally irradiated mice, *J. Immunol.* **115:**844–847.
254. Cutler, J. E., 1976, Acute systemic candidiasis in normal and congenitally thymic-deficient (nude) mice, *J. Reticuloendothel. Soc.* **19:**121–124.
255. Poor, A. H., and Cutler, J.E ., 1981, Analysis of an *in vivo* model to study the interaction of host factors with *Candida albicans*, *Infect. Immun.* **31:**1104–1109.
256. Bistoni, F., Vecchiarelli, A., Cenci, E., Puccetti, P., Marconi, P., and Cassone, A., 1986, Evidence for macrophage-mediated protection against lethal *Candida albicans* infection, *Infect. Immun.* **51:**668–674.
257. Chow, H. S., Sarpel, S. C., and Epstein, R. B., 1980, Pathophysiology of *Candida albicans* meningitis in normal, neutropenic and granulocyte transfused dogs, *Blood* **55:**546–551.
258. Bistoni, F., Baccarini, M., Blasi, E., Marconi, P., Puccetti, P., and Garaci, E., 1983, Correlation between *in vivo* and *in vitro* studies of modulation of resistance to experimental *Candida albicans* infection by cyclophosphamide in mice, *Infect. Immun.* **40:**46–55.
259. Fromtling, R. A., Abruzzo, G. K., and Giltinan, D. M., 1987, *Candida tropicalis* infection in normal, diabetic, and neutropenic mice, *J. Clin. Microbiol.* **25:**1416–1420.
260. van't Wout, J. W., Linde, I., Leijh, P. C. J., and van Furth, R., 1988, Contribution of granulocytes and monocytes to resistance against experimental disseminated *Candida albicans* infection, *Eur. J. Clin. Microbiol. Infect. Dis.* **7:**736–741.
261. Sawyer, R. T., and Harmsen, A. G., 1989, The relative contribution of resident pulmonary alveolar macrophage and inflammatory polymorphonuclear neutrophils in host resistance to pulmonary infection by *Candida albicans*, *Mycopathologia* **108:**95–105.
262. Oblack, D. L., Schwarz, J., and Holder, I. A., 1979, Enhancement of tissue damage by *Candida albicans* in cortisone or nitrogen mustard-treated mice, *Can. J. Microbiol.* **25:**1015–1020.
263. Nugent, K. M., and Onofrio, J. M., 1984, Pulmonary clearance of *Candida albicans* in neutropenic mice, *J. Infect. Dis.* **149:**1020.

264. Hasenclever, H. F., and Corley, E. J., 1968, Enhancement of acquired resistance in murine candidiasis by *Bordetella pertussis* vaccine, *Sabouraudia* **6:**289–295.
265. Marra, S., and Balish, E., 1974, Immunity to *Candida albicans* induced by *Listeria monocytogenes*, *Infect. Immun.* **10:**72–82.
266. Sher, N. A., Chaparas, S. D., Greenberg, L. E., and Bernard, S., 1975, Effects of BCG, *Corynebacterium parvum*, and methanol-extraction residue in the reduction of mortality from *Staphylococcus aureus* and *Candida albicans* infection in immunosuppressed mice, *Infect. Immun.* **12:**1325–1330.
267. Rogers, T. J., and Balish, E., 1977, The role of activated macrophages in resistance to experimental renal candidiasis, *J. Reticuloendothel. Soc.* **22:**309–318.
268. Sawyer, R. T., Moon, R. J., and Beneke, E. S., 1981, Trapping and killing of *Candida albicans* by *Corynebacterium parvum*-activated livers, *Infect. Immun.* **32:**945–950.
269. Cummings, N. P., Pabst, M. J., and Johnston, R. B., Jr., 1980, Activation of macrophages for enhanced release of superoxide anion and greater killing of *Candida albicans* by injection of muramyl dipeptide, *J. Exp. Med.* **152:**1659–1669.
270. Fraser-Smith, E. B., Waters, R. V., and Matthews, T. R., 1982, Correlation between *in vivo* anti-*Pseudomonas* and anti-*Candida* activities and clearance of carbon by the reticuloendothelial system for various muramyl dipeptide analogs, using normal and immunosuppressed mice, *Infect. Immun.* **35:**105–110.
271. Fraser-Smith, E. B., Eppstein, D. A., Larsen, M. A., and Matthews, T. R., 1983, Protective effect of a muramyl dipeptide analog encapsulated in or mixed with liposomes against *Candida albicans* infection, *Infect. Immun.* **39:**172–178.
272. Baccarini, M., Blasi, E., Puccetti, P., and Bistoni, F., 1983, Phagocytic killing of *Candida albicans* by different murine effector cells, *Sabouraudia* **21:**271–286.
273. Baccarini, M., Bistoni, F., and Lohmann-Matthes, M. L., 1986, Organ-associated macrophage precursor activity: isolation of candidacidal and tumoricidal effectors from the spleens of cyclophosphamide-treated mice, *J. Immunol.* **136:**837–843.
274. Decker, T., Baccarini, M., and Lohmann-Matthes, M. L., 1986, Liver-associated macrophage precursors as natural cytotoxic effectors against *Candida albicans* and Yac-1 cells, *Eur. J. Immunol.* **16:**693–699.
275. Baccarini, M., Bistoni, F., and Lohmann-Matthes, M.-L., 1985, *In vitro* natural cell-mediated cytotoxicity against *Candida albicans*: macrophage precursors as effector cells, *J. Immunol.* **134:**2658–2665.
276. Decker, T., Lohmann-Matthes, M. L., and Baccarini, M., 1986, Heterogeneous activity of immature and mature cells of the murine monocyte-macrophage lineage derived from different anatomical districts against yeast-phase *Candida albicans*, *Infect. Immun.* **54:** 477–486.
277. Meister, H., Heymer, B., Schafer, H., and Haferkamp, O., 1977, Role of *Candida albicans* in granulomatous tissue reactions. II. *In vivo* degradation of *C. albicans* in hepatic macrophages of mice, *J. Infect. Dis.* **135:**235–242.
278. Lehrer, R. I., Ferrari, L. G., Patterson-Delafield, J., and Sorrell, T., 1980, Fungicidal activity of rabbit alveolar and peritoneal macrophages against *Candida albicans*, *Infect. Immun.* **28:** 1001–1008.
279. Thaler, M., Pastakia, B., Shawker, T. H., O'Leary, T., Pizzo, P. A., 1988, Hepatic candidiasis in cancer patients: the evolving picture of the syndrome, *Ann. Intern. Med.* **108:**88–100.
280. Carstensen, H., Widding, E., Storm, K., Ostergaard, E., Herlin, T., 1990, Hepatosplenic candidiasis in children with cancer. Three cases in leukemia children and a literature review, *Pediatr. Hematol. Oncol.* **7:**3–12.
281. Pizzo, P. A., and Walsh, T. J., 1990, Fungal infections in the pediatric cancer patient. *Semin. Oncol.* **17:**6–9.
282. Damodaran, V. N., and Chakravarty, S. C., 1973, Mechanism of production of *Candida* lesions in rabbits, *J. Med. Microbiol.* **6:**287–292.

283. Peterson, E. M., and Calderone, R. A., 1977, Growth inhibition of *Candida albicans* by rabbit alveolar macrophages, *Infect. Immun.* **15**:910–915.
284. Peterson, E. M., and Calderone, R. A., 1978, Inhibition of specific amino acid uptake in *Candida albicans* by lysosomal extracts from rabbit alveolar macrophages, *Infect. Immun.* **21**: 506–513.
285. Patterson-Delafield, J., Szklarek, D., Martinez, R. J., and Lehrer, R. I., 1981, Microbicidal cationic proteins of rabbit alveolar macrophages: amino acid composition and functional attributes, *Infect. Immun.* **31**:723–731.
286. Selsted, M. E., Brown, D. M., DeLange, R. J., and Lehrer, R. I., 1983, Primary structures of MCP-1 and MCP-2, natural peptide antibiotics of rabbit lung macrophages, *J. Biol. Chem.* **258**:14485–14489.
287. Ganz, T., Sherman, M. P., Selsted, M. E., and Lehrer, R. I., 1985, Newborn rabbit alveolar macrophages are deficient in two microbicidal cationic peptides, MCP-1 and MCP-2, *Am. Rev. Respir. Dis.* **132**:901–904.
288. D'Ambola, J. B., Sherman, M. P., Tashkin, D. P., Gong, H., Jr., 1988, Human and rabbit newborn lung macrophages have reduced anti-*Candida* activity, *Pediatr. Res.* **24**:285–290.
289. LaForce, F. M., Sharrar, R. G., and Arai, G., 1979, Characterization of yeast agglutinins in lavage fluid from lungs of rabbits, *J. Infect. Dis.* **140**:96–104.
290. Arai, T., Mikami, Y., and Yokoyama, K., 1977, Phagocytosis of *Candida albicans* by rabbit alveolar macrophages and guinea pig neutrophils, *Sabouraudia* **15**:171–177.
291. Gontijo, P. P., Jr., and Wayne, L. G., 1978, Phagocytic and antimicrobial activities of rabbit peritoneal and alveolar macrophages activated with *Mycobacterium bovis* BCG on *Candida albicans*, *Rev. Latinoam. Microbiol.* **20**:41–44.
292. Evans, Z. A., 1980, Tissue responses to the blastospores and hyphae of *Candida albicans* in the mouse, *J. Med. Microbiol.* **14**:307–319.
293. Nugent, K. M., and Onofrio, J. M., 1983, Pulmonary tissue resistance to *Candida albicans* in normal and in immunosuppressed mice, *Am. Rev. Respir. Dis.* **128**:909–914.
294. Lal, S., Mitsuyama, M., Amako, K., and Nomoto, K., 1986, Enhanced clearance of *Candida albicans* from lung after intratracheal immunization, *J. Clin. Lab. Immunol.* **21**:23–30.
295. Lal, S., Mitsuyama, M., Miyata, M., Ogata, N., Amako, K., and Nomoto, K., 1986, Pulmonary defense mechanism in mice. A comparative role of alveolar macrophages and polymorphonuclear cells against infection with *Candida albicans*, *J. Clin. Lab. Immunol.* **19**: 127–133.
296. Nugent, K. M., and Fick, R. B., Jr., 1987,Candidacidal factors in murine bronchoalveolar lavage fluid, *Infect. Immun.* **55**:541–546.
297. Warr, G. A., and Jakab, G. J., 1979, Alterations in lung macrophage antimicrobial activity associated with viral pneumonia, *Infect. Immun.* **26**:492–497.
298. Kurland, G., Cheung, A. T., Miller, M. E., Ayin, S. A., Cho, M. M., and Ford, E. W., 1988, The ontogeny of pulmonary defenses: alveolar macrophage function in neonatal and juvenile rhesus monkeys, *Pediatr. Res.* **23**:293–297.
299. Brummer, E., and Stevens, D. A., 1987, Activation of pulmonary macrophages for fungicidal activity by gamma-interferon or lymphokines, *Clin. Exp. Immunol.* **70**:520–528.
300. Stanley, V. C., and Hurley, R., 1969, The growth of *Candida* species in cultures of mouse peritoneal macrophages, *J. Pathol.* **97**:357–366.
301. Ozato, K., and Uesaka, I., 1974, The role of macrophages in *Candida albicans* infection *in vitro*, *Jap. J. Microbiol.* **18**:29–35.
302. Sasada, M., and Johnston, R. B., Jr., 1980, Macrophage microbicidal activity: correlation between phagocytosis-associated oxidative metabolism and the killing of *Candida* by macrophages, *J. Exp. Med.* **152**:85–98.
303. Nozawa, R. T., Sekiguchi, R., and Yokota, T., 1980, Stimulation by conditioned medium of L-929 fibroblasts, *E. coli* lipopolysaccharide, muramyl dipeptide of candidacidal activity of mouse macrophages, *Cell. Immunol.* **53**:116–124.

304. Maiti, P. K., Kumar, R., and Mohapatra, L. N., 1980, Candidacidal activity of mouse macrophages *in vitro*, *Infect. Immun.* **29:**477–482.
305. Mor, N., and Goren, M. B., 1987,Discrepancy in assessment of phagosome-lysosome fusion with two lysosomal markers in murine macrophages infected with *Candida albicans*, *Infect. Immun.* **55:**1663–1667.
306. Brummer, E., Morrison, C. J., and Stevens, D. A., 1985, Recombinant and natural gamma-interferon activation of macrophages *in vitro*: different dose requirements for induction of killing activity against phagocytizable and nonphagocytizable fungi, *Infect. Immun.* **49:** 724–730.
307. Kolotila, M. P., Smith, C. W., and Rogers, A. L., 1987, Candidacidal activity of macrophages from three mouse strains as demonstrated by a new method: neutral red staining, *J. Med. Vet. Mycol.* **25:**283–290.
308. Lehrer, R. I., Ferrari, L. G., and Patterson-Delafield, J., 1977, The fungicidal activity of rabbit macrophages, *Clin. Res.* **25:**490A.
309. Kagaya, K., and Fukazawa, Y., 1981, Murine defense mechanisms against *Candida albicans* infection. II. Opsonization, phagocytosis and intracellular killing of *C. albicans*, *Microbiol. Immunol.* **25:**807–818.
310. Brummer, E., and Stevens, D. A., 1989, Candidacidal mechanisms of peritoneal macrophages activated with lymphokines or gamma-interferon, *J. Med. Microbiol.* **28:**173–181.
311. Diamond, R. D., and Haudenschild, C. C., 1981, Monocyte-mediated serum independent damage to hyphal and pseudohyphal forms of *Candida albicans in vitro*, *J. Clin. Invest.* **67:** 173–182.
312. Patterson-Delafield, J., Martinez, R. J., and Lehrer, R. I., 1980, Microbicidal cationic proteins in rabbit alveolar macrophages: a potential host defense mechanism, *Infect. Immun.* **30:**180–192.
313. Lehrer, R. I., Szklarek, D., Selsted, M. E., and Fleischmann, J., 1981, Increased content of microbicidal cationic peptides in rabbit alveolar macrophages elicited by complete Freund adjuvant, *Infect. Immun.* **33:**775–778.
314. Mourad, S., and Friedman, L., 1961, Active immunization of mice against *Candida albicans*, *Proc. Soc. Exp. Biol. Med.* **106:**570–572.
315. Oblack, D. L., and Holder, I. A., 1979, Active immunization of mice against muscle damage mediated by *Candida albicans*, *J. Med. Microbiol.* **12:**503–506.
316. Banerjee, U., Mohapatra, L. N., and Kumar, R., 1985, Effect of immunization with formalin killed cells in complete Freund's adjuvant in experimental candidosis, *Indian J. Med. Res.* **81:**454–458.
317. Levy, R., Segal, E., and Eylan, E., 1981, Protective immunity against murine candidiasis elicited by *Candida albicans* ribosomal fractions, *Infect. Immun.* **31:**874–878.
318. Kuttin, E. S., Muller, J., Douchet, C., and Vogt, A., 1983, Immunological and pathological observations with *Candida albicans*-infected animals, *Sabouraudia* **21:**185–194.
319. Hasenclever, H. F., and Mitchell, W. O., 1963, Acquired immunity to candidiasis in mice, *J. Bacteriol.* **86:**401–406.
320. Giger, D. K., Domer, J. E., and McQuitty, J. T., Jr., 1978, Experimental murine candidiasis: pathologic and immune responses to cutaneous inoculation with *Candida albicans*, *Infect. Immun.* **19:**499–509.
321. 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.
322. Mourad, S., and Friedman, L., 1968, Passive immunization of mice against *Candida albicans*, *Sabouraudia* **6:**103–105.
323. Al-Doory, Y., 1970, An immune factor in baboon anti-*Candida* serum, *Sabouraudia* **8:** 41–47.
324. Pearsall, N. N., Adams, B. L., and Bunni, R., 1978, Immunologic responses to *Candida*

albicans. III. Effect of passive transfer of lymphoid cells or serum on murine candidiasis, *J. Immunol.* **120**:1176–1180.

325. Moser, S. A., and Domer, J. E., 1980, Effects of cyclophosphamide on experimental murine candidiasis, *Infect. Immun.* **27**:376–386.
326. 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-μ, *J. Leukoc. Biol.* **44**:422–433.
327. 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**:263–266.
328. Domer, J. E., and Hector, R. H., 1987, Enhanced immune responses in mice treated with penicillin/tetracycline or trimethoprim-sulfamethoxazole when colonized intragastrically with *Candida albicans*, *Antimicrob. Agents Chemother.* **31**:691–697.
329. Hurd, R. C., and Drake, C. H., 1953, *Candida albicans* infections in actively and passively immunized animals, *Mycopathol. Mycol. Appl.* **6**:290–297.
330. Miyake, T., Takeya, K., Nomoto, K., and Muraoka, S., 1977, Cellular elements in the resistance to *Candida* infection in mice. I. Contribution of T lymphocytes and phagocytes at various stages of infection, *Microbiol. Immunol.* **21**:703–725.
331. Banerjee, U., Mohapatra, L. N., and Kumar, R., 1984, Role of antibody in defence against murine candidosis, *Indian J. Med. Res.* **79**:760–765.
332. Giger, D. K., Domer, J. E., Moser, S. A., and McQuitty, J. T., Jr., 1978, Experimental murine candidiasis: pathological and immune responses in T-depleted mice, *Infect. Immun.* **21**: 729–737.
333. Grubek, H., Szymanska, D., Trenkner, E,. Weyman-Rzucidlo, D., and Okon, M., 1970, Effect of anti-lymphocyte serum on experimental infection with *Candida albicans* in guinea pigs, *Acta Microbiol. Pol. Ser.* A **2**:21–30.
334. Sohnle, P. G., Frank, M. M., and Kirkpatrick, C. H., 1976, Mechanisms involved in elimination of organisms from experimental cutaneous *Candida albicans* infections in guinea pigs, *J. Immunol.* **117**:523–530.
335. Cenci, E., Romani, L., Vecchiarelli, A., Puccetti, P., and Bistoni, F., 1989, Role of L3T4+ lymphocytes in protective immunity to systemic *Candida albicans* infection in mice, *Infect. Immun.* **59**:3581–3587.
336. Cenci, E., Romani, L., Vecchiarelli, A., Puccetti, P., and Bistoni, F., 1990, T cell subsets and IFN-gamma production in resistance to systemic candidosis in immunized mice, *J. Immunol.* **144**:4333–4339.
337. Vecchiarelli, A., Cenci, E., Puliti, M., Blasi, E., Puccetti, P., Cassone, A., and Bistoni, F., 1989, Protective immunity induced by low-virulence *Candida albicans*: cytokine production in the development of the anti-infectious state, *Cell. Immunol.* **124**:334–344.
338. Garner, R. E., Kuruganti, U., Czarniecki, C. W., Chiu, H. H., and Domer, J. E., 1989, *In vivo* immune responses to *Candida albicans* modified by treatment with recombinant murine gamma interferon, *Infect. Immun.* **57**:1800–1808.
339. Sen, P., Smith, J. K., Buse, M., Hsieh, H. C., Lavenhar, M. A., Lintz, D., and Louria, D. B., 1982, Modification of an experimental mouse *Candida* infection by human dialyzable leukocyte extract, *Sabouraudia* **20**:85–93.
340. Kagaya, K., Shinoda, R., and Fukazawa, Y., 1981, Murine defense mechanisms against *Candida albicans* infection. I. Collaboration of cell-mediated and humoral immunities in protection against systemic *C. albicans* infection, *Microbiol. Immunol.* **25**:647–654.
341. Sinha, B. K., Prasad, S., and Monga, D. P., 1987, Studies of experimental candidiasis in T-cell-deficient mice, *Zbt. Bacteriol. Hyg.* **A 256**:203–209.
342. Ashman, R. B., 1990, Murine candidiasis: cell-mediated immune responses correlate directly with susceptibility and resistance to infection, *Immunol. Cell Biol.* **68**:15–20.
343. Hurtrel, B., Lagrange, P. H., and Michel, J.-C., 1981, Absence of correlation between delayed-type hypersensitivity and protection in experimental systemic candidiasis in immunized mice, *Infect. Immun.* **31**:95–101.

344. Salvin, S. B., Peterson, R. D. A., and Good, R. A., 1965, The role of the thymus in resistance to infection and endotoxin toxicity, *J. Lab. Clin. Med.* **65:**1004–1022.
345. Gordee, R. S., and Simpson, P. J., 1967, Relationships of x-irradiation to the enhancement of *Candida albicans* infections, *J. Bacteriol.* **94:**6–12.
346. Rogers, T. J., Balish, E., and Manning, D. D., 1976, The role of thymus-dependent cell-mediated immunity in resistance to experimental disseminated candidiasis, *J. Reticuloendothel. Soc.* **20:**291–298.
347. Ashman, R. B., 1987, Mouse candidiasis. II. Host responses are T-cell dependent and regulated by genes in the major histocompatibility complex, *Immunogenetics* **25:** 200–203.
348. Sinha, B. K., Prasad, S., and Monga, D. P., 1987, Studies on the role of B-cells in the resistance of mice to experimental candidiasis, *Zbl. Bakt. Hyg.* **A 266:**316–322.
349. Mahanty, S., Greenfield, R. A., Joyce, W. A., and Kincade, P. W., 1988, Inoculation candidiasis in a murine model of severe combined immunodeficiency syndrome, *Infect. Immun.* **56:**3162–3166.
350. Marquis, G., Montplaisir, S., Pelletier, M., Auger, P., and Lapp, W. S., 1988, Genetics of resistance to infection with *Candida albicans* in mice, *Br. J. Exp. Path.* **69:**651–660.
351. Vecchiarelli, A., Cenci, E., Marconi, P., Rossi, R., Riccardi, C., and Bistoni, F., 1989, Immunosuppressive effect of cyclosporin A on resistance to systemic infection with *Candida albicans*, *J. Med. Microbiol.* **30;**183–192.
352. Bistoni, F., Marconi, P., Frati, L., Bonmassar, E., and Garcia, E., 1982, Increase of mouse resistance to *Candida albicans* infection by thymosin a1, *Infect. Immun.* **36:**609–614.
353. Marquis, G., Montplaisir, S., Pelletier, M., Mousseau, S., and Auger, P., 1986, Strain-dependent differences in susceptibility of mice to experimental candidosis, *J. Infect. Dis.* **154:**906–909.
354. Ashman, R. B., and Papadimitriou, J. M., 1987, Murine candidiasis. Pathogenesis and host responses in genetically distinct inbred mice, *Immunol. Cell Biol.* **65:**163–171.
355. Ashman, R. B., 1990, Murine candidiasis: susceptibility is associated with the induction of T cell-mediated, strain-specific autoreactivity, *Immunol. Cell Biol.* **68:**179–185.
356. Ashman, R. B., 1987, Murine candidiasis. III. Host inflammatory responses are regulated in part by class I MHC genes, *J. Immunogenet.* **14:**317–321.
357. Ashman, R. B., and Papadimitriou, J. M., 1989, Genetic regulation of pathogenesis and host responses in fungal infection, in *Immunology of Fungal Diseases* (E. Kurstak, ed.), Marcel Dekker, Inc., New York, pp. 347–371.
358. Fischer, A., Ballet, J. J., and Griscelli, C., 1978, Specific inhibition of in vitro *Candida*-induced lymphocyte proliferation by polysaccharidic antigens present in serum of patients with chronic mucocutaneous candidiasis, *J. Clin. Invest.* **62:**1005–1013.
359. Witkin, S. S., Yu, R., and Ledger, W. J., 1983, Inhibition of *Candida albicans*-induced lymphocyte proliferation by lymphocytes and sera from women with recurrent vaginitis, *Am. J. Obstet. Gynecol.* **147:**809–811.
360. Borkowsky, W., and Valentine, F. T., 1979, The proliferative response of human lymphocytes to antigen is suppressed preferentially by lymphocytes precultured with the same antigen, *J. Immunol.* **122:**1867–1873.
361. Rogers, T. J., and Balish, E., 1978, Effect of systemic candidiasis on blastogenesis of lymphocytes from germfree and conventional rats, *Infect. Immun.* **20:**142–150.
362. Skerl, K. G., Scheld, W. M., Alliegro, G. M., and Calderone, R. A., 1980, Lymphocyte blastogenesis during experimental endocarditis caused by *Candida albicans*, *J. Reticuloendothel. Soc.* **28:**495–506.
363. Piccolella, E., Lombardi, G., and Morelli, R., 1981, Generation of suppressor cells in the response of human lymphocytes to a polysaccharide from *Candida albicans*, *J. Immunol.* **126:** 2151–2159.
364. Rivas, V., and Rogers, T. J., 1983, Studies on the cellular nature of *Candida albicans*-induced suppression, *J. Immunol.* **130:**376–379.

365. Cuff, C. F., Rogers, C. M., Lamb, B. J., and Rogers, T. J., 1986, Induction of suppressor cells in vitro by *Candida albicans*, *Cell. Immunol.* **100:**47–56.
366. Nelson, R. D., Herron, M. J., McCormack, R. T., and Gehrz, R. C., 1984, Two mechanisms of inhibition of human lymphocyte proliferation by soluble yeast mannan polysaccharide, *Infect. Immun.* **43:**1041–1046.
367. Witkin, S. S., 1986, Inhibition of *Candida*-induced lymphocyte proliferation by antibody to *Candida albicans*, *Obstet. Gynecol.* **68:**696–699.
368. Podzorski, R. P., Herron, M. J., Fast, D. J., and Nelson, R. D., 1989, Pathogenesis of candidiasis. Immunosuppression by cell wall mannan catabolites, *Arch. Surg.* **124:**1290–1294.
369. Podzorski, R. P., Gray, G. R., and Nelson, R. D., 1990, Different effects of native *Candida albicans* mannan and mannan-derived oligosaccharides on antigen-stimulated lymphoproliferation in vitro, *J. Immunol.* **144;**707–716.
370. Cuff, C. F., Packer, B. J., and Rogers, T. J., 1989, A further characterization of *Candida albicans*-induced suppressor B-cell activity, *Immunology* **68:**80–86.
371. Cuff, C. F., Taub, D. D., and Rogers, T. J., 1989, The induction of T-suppressor cells with a soluble extract of *Candida albicans*, *Cell. Immunol.* **122:**71–82.
372. Segal, E., Schwartz, J., Altboun, Z., Vardinon, N., and Eylan, E., 1977, Suppressive action of *Candida albicans* on the immune response in guinea pigs, *Microbios* **19:**79–87.
373. Vardinon, N., and Segal, E., 1979, Suppressive action of *Candida albicans* on the immune response in mice, *Exp. Cell Biol.* **47:**275–280.
374. Segal, E., Sandovsky-Losica, H., and Vardinon, N., 1980, Suppressive action of cytoplasmic and metabolic extracts of *Candida albicans* on the immune response in guinea pigs, *Mycopathologia* **72:**121–128.
375. Cutler, J. E., and Lloyd, R. K., 1982, Enhanced antibody responses induced by *Candida albicans* in mice, *Infect. Immun.* **38:**1102–1108.
376. 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:**172–181.
377. Domer, J. E., Stashak, P. W., Elkins, K., Prescott, B., Caldes, G., and Baker, P. J., 1986, Separation of immunomodulatory effects of mannan from *Candida albicans* into stimulatory and suppressive components, *Cellular Immunol.* **101:**403–414.
378. Domer, J. E., Elkins, K. L., Ennist, D. L., Stashak, P. W., Garner, R. E., and Baker, P. J., 1987, Immunoenhancement of non-*Candida* antibody responses by *Candida albicans* cell wall glycoprotein, *Infect. Immun.* **55:**2619–2624.
379. Domer, J., Garner, R., and Befidi-Mengue, R., 1989, Mannan as an antigen in cell-mediated immune (CMI) assays and as a modulator of mannan-specific CMI, *Infect. Immun.* **57:** 693–700.
380. Garner, R. E., Childress, A. M., Human, L. G., and Domer, J. E., 1990, Characterization of *Candida albicans* mannan-induced, mannan-specific delayed hypersensitivity suppressor cells, *Infect. Immun.* **58:**2613–2620.
381. Piccolella, E., Lombardi, G., and Morelli, R., 1980, Human lymphocyte-activating properties of a purified polysaccharide from *Candida albicans*: B and T cell cooperation in the mitogenic response, *J. Immunol.* **125:**2082–2088.
382. Lombardi, G., Vismara, D., Piccolella, E., Colizzi, V., and Asherson, G. L., 1985, A non-specific inhibitor produced by *Candida albicans* activated T cells impairs cell proliferation by inhibiting interleukin-1 production, *Clin. Exp. Immunol.* **60:**303–310.
383. Peat, S., Whelan, W. J., and Edwards, T. E., 1961, Polysaccharide of baker's yeast. Part IV. Mannan, *J. Chem. Soc.* (London) **1:**29–34.
384. Durandy, A., Fischer, A., Charron, D., and Griscelli, G., 1986, Specific binding of antigen onto human T lymphocytes, *J. Clin. Invest.* **77:**1557–1564.
385. Fischer, A., Pichat, L., Audinot, M., and Griscelli, C., 1982, Defective handling of mannan

by monocytes in patients with chronic mucocutaneous candidiasis resulting in a specific cellular unresponsiveness, *Clin. Exp. Immunol.* **47**:653–660.
386. Wright, C. D., Herron, M. J., Gray, G. R., Holmes, B., and Nelson, R. D., 1981, Influence of yeast mannan on human neutrophil functions: inhibition of release of myeloperoxidase related to carbohydrate-binding property of the enzyme, *Infect. Immun.* **32**:731–738.
387. Winner, H. I., and Hurley, R., 1964, *Candida Albicans*, J. & A. Churchill Ltd, London.
388. Taschdjian, D. L., Kozinn, P. J., and Caroline, L., 1964, Immune studies in candidiasis. III. Precipitating antibodies in systemic candidiasis, *Sabouraudia* **3**:312–320.
389. de Repentigny, L., and Reiss, E., 1984, Current trends in immunodiagnosis of candidosis and aspergillosis, *Rev. Infect. Dis.* **6**:301–312.
390. Bennett, J. E., 1987, Rapid diagnosis of candidiasis and aspergillosis, *Rev. Infect. Dis.* **9**: 398–402.
391. de Repentigny, L., 1989, Serological techniques for diagnosis of fungal infection, *Eur. J. Clin. Microbiol. Infect. Dis.* **81**:362–375.
392. Evans, E. G. V., Richardson, M. D., Odds, F. C., and Holland, K. T., 1973, Relevance of antigenicity of *Candida albicans* growth phases to diagnosis of systemic candidiasis, *Br. Med. J.* **4**:86–87.
393. Syverson, R. E., Buckley, H. R., and Campbell, C. C., 1975, Cytoplasmic antigens unique to the mycelial or yeast phase of *Candida albicans*, *Infect. Immun.* **12**:1184–1188.
394. Manning, M., and Mitchell, T. G., 1980, Morphogenesis of *Candida albicans* and cytoplasmic proteins associated with differences in morphology, strain, or temperature, *J. Bacteriol.* **144**: 258–273.
395. Ho, Y. M., Ng, M. H., and Huang, C. T., 1979, Antibodies to germinating and yeast cells of *Candida albicans* in human and rabbit sera, *J. Clin. Pathol.* **32**:399–405.
396. Smail, E. H., and Jones, J. M., 1984, Demonstration and solubilization of antigens expressed primarily on the surfaces of *Candida albicans* germ tubes, *Infect. Immun.* **45**:74–81.
397. 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**: 850–855.
398. Ponton, J., and Jones, J. M., 1986, Analysis of cell wall extracts of *Candida albicans* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western blot techniques, *Infect. Immun.* **53**:565–572.
399. Brawner, D. J., and Cutler, J. E., 1984, Variability in expression of a cell surface determinant as evidenced by an agglutinating monoclonal antibody, *Infect. Immun.* **43**:966–972.
400. Brawner, D. J., and Cutler, J. E., 1986, Variability in expression of cell surface antigens of *Candida albicans* during morphogenesis, *Infect. Immun.* **51**;337–343.
401. Chaffin, W. L., Skudlarek, J., and Morrow, K. J., 1988, Variable expression of a surface determinant during proliferation of *Candida albicans*, *Infect. Immun.* **56**:302–309.
402. Summers, D. F., Grollman, A. P., and Hasenclever, H. F., 1964, Polysaccharide antigens of *Candida* cell wall, *J. Immunol.* **92**:491–499.
403. Syverson, R. E., and Buckley, H. R., 1977, Cell wall antigens in soluble cytoplasmic extracts of *Candida albicans* as demonstrated by crossed-immunoelectrophoresis with concanavalin A, *J. Immunol. Methods* **18**:149–156.
404. Munoz, M., Estes, G., Kilpatrick, M., DiSalvo, A., and Virella, G., 1980, Purification of cytoplasmic antigens from the mycelial phase of *Candida albicans*: possible advantages of its use in *Candida* serology, *Mycopathologia* **72**:47–53.
405. Taschdjian, C. L., Dobbin, G. B., Kozinn, P. J., and Caroline, L., 1964, Experimental and preliminary clinical studies on antibody formation in systemic candidiasis, *Sabouraudia* **3**: 129–139.
406. Remington, J. S., Gaines, J. D., and Gilmer, M. A., 1972, Demonstration of *Candida* precipitins in human sera by counter-immunoelectrophoresis, *Lancet* **1**:413.
407. Axelsen, N. H., Buckley, H. R., Drouhet, E., Budtz-Jorgensen, E., Hattel, T., and Andersen,

P. L., 1975, Crossed immunoelectrophoretic analysis of precipitins to *Candida albicans* in deep *Candida* infection, *Scand. J. Immunol.* **4:**217–230.

408. Lehmann, P. F., and Reiss, E., 1980, Comparison by ELISA of serum anti-*Candida albicans* mannan IgG levels of a normal population and in diseased patients, *Mycopathologia* **70:** 89–93.
409. Meckstroth, K. L., Reiss, E., Keller, J. W., and Kaufman, L., 1981, Detection of antibodies and antigenemia in leukemic patients with candidiasis by enzyme-linked immunosorbent assay, *J. Infect. Dis.* **144:**24–32.
410. Odds, F. C., Evans, E. G. V., and Holland, K. T., 1975, Detection of *Candida* precipitins. A comparison of double diffusion and counter immunoelectrophoresis, *J. Immunol. Methods* **7:** 211–218.
411. Harding, S. A., Sanford, G. R., and Merz, W. G., 1976, Three serologic tests for candidiasis. Diagnostic value in distinguishing deep or disseminated infection from superficial infection or colonization, *Am. J. Clin. Pathol.* **65:**1001–1009.
412. Dee, T. H., and Rytel, M. W., 1977, Detection of *Candida* serum precipitins by counter-immunoelectrophoresis: an adjunct in determining significant candidiasis, *J. Clin. Microbiol.* **5:**453–457.
413. Merz, W. G., Evans, G. L., Shadomy, S., Anderson, S., Kaufman, L., Kozinn, P. J., Mackenzie, D. W., Protzman, W. P., and Remington, J. S., 1977, Laboratory evaluation of serological tests for systemic candidiasis: a cooperative study, *J. Clin. Microbiol.* **5:**596–603.
414. Kozinn, P. J., Taschdjian, C. L., Goldberg, P. K., Protzmann, W. P., Mackenzie, D. W. R., Remington, J. S., Anderson, S., and Seelig, M. S., 1978, Efficacy of serological tests in the diagnosis of systemic candidiasis, *Am. J. Clin. Pathol.* **70:**893–898.
415. Dee, T. H., Johnson, G. M., and Berger, C. S., 1981, Sensitivity specificity, and predictive value of anti-*Candida* serum precipitin and agglutinin quantification: comparison of counterimmunoelectrophoresis and latex agglutination, *J. Clin. Microbiol.* **13:**750–753.
416. Bisbe, J., Miro, J. M., Torres, J. M., Latorre, X., Alia, C., Amaral, M., Estivill, D., Mallalas, J., Trilla, A., and Soriano, E., 1989, Diagnostic value of serum antibody and antigen detection in heroin addicts with systemic candidiasis, *Rev. Infect. Dis.* **11:**310–315.
417. Syverson, R. E., Buckley, H. R., and Gibian, B. A., 1978, Increasing the predictive value positive of the precipitin test for the diagnosis of deep-seated candidiasis, *Am. J. Clin. Pathol.* **70:**826–831.
418. Glew, R. H., Buckley, H. R., Rosen, H. M., Moellering, R. C., and Fischer, J. E., 1978, Serologic tests in the diagnosis of systemic candidiasis. Enhanced diagnostic accuracy with crossed immunoelectrophoresis, *Am. J. Med.* **64:**586–591.
419. Poulain, D., and Pinon, J. M., 1986, Diagnosis of systemic candidiasis: development of co-counterimmunoelectrophoresis, *Eur. J. Clin. Microbiol.* **5:**420–426.
420. Poulain, D., and Pinon, J. M., 1986, Application of co-counterimmunoelectrophoresis, *Eur. J. Clin Microbiol.* **5:**427–434.
421. Bougnoux, M.-E., Hill, C., Moissenet, D., de Chavin, M. F., Bonnay, M., Vicens-Sprauel, I., Pietri, F., McNeil, M., Kaufman, L., Dupouy-Camet, J,. Bohuon, C., and Andremont, A., 1990, Comparison of antibody, antigen, and metabolite assays for hospitalized patients with disseminated or peripheral candidiasis, *J. Clin. Microbiol.* **28:**905–909.
422. Jones, J. M., 1980, Quantitation of antibody against cell wall mannan and a major cytoplasmic antigen of *Candida* in rabbits, mice and humans, *Infect. Immun.* **30:**78–89.
423. Matthews, R. C., Burnie, J. P., and Tabaqchali, S., 1984, Immunoblot analysis of the serologic response in systemic candidosis, *Lancet* **2:**1415–1418.
424. Au-Young, J. K., Troy, F. A., and Goldstein, E., 1985, Serologic analysis of antigen-specific reactivity in patients with systemic candidiasis, *Diag. Microbiol. Infect. Dis.* **3:**419–432.
425. Gatermann, S., Heesemann, J., and Laufs, R., 1986, Identification of *Candida albicans* antigens recognized by sera of patients with candidiasis, *Mykosen* **29:**343–354.
426. Strockbine, N. A., Largen, M. T., Zweibel, S. M., and Buckley, H. R., 1984, Identification

and molecular weight characterization of antigens from *Candida albicans* that are recognized by human sera, *Infect. Immun.* **43:**715-721.

427. Porsius, J. C., van Vleit, H. J., van Zeijl, J. H., Goessens, W. H., and Michel, M. F., 1990, Detection of an antibody response in immunocompetent patients with systemic candidiasis or *Candida albicans* colonization, *Eur. J. Clin. Microbiol. Infect. Dis.* **9:**352–355.
428. Greenfield, R. A., and Jones, J. M., 1981, Purification and characterization of a major cytoplasmic antigen of *Candida albicans*, *Infect. Immun.* **34:**469–477.
429. Fiss, E., and Buckley, H. R., 1987, Purification of actin from *Candida albicans* and comparison with the *Candida* 48,000-Mr Protein, *Infect. Immun.* **55:**2324–2326.
430. Mason, A. B., Brandt, M. E., and Buckley, H. R., 1989, Enolase activity associated with *Candida albicans* cytoplasmic antigen, *Yeast* **5:**231–239.
431. Franklyn, K. M., Warmington, J. R., Ott, A. K., and Ashman, R. B., 1990, An immunodominant antigen of *Candida albicans* shows homology to the enzyme enolase, *Immunol. Cell Biol.* **68:**173–178.
432. Matthews, R. C., Burnie, J. P., and Tabaqchali, S., 1987, Isolation of immunodominant antigens from sera of patients with systemic candidiasis and characterization of serological response to *Candida albicans*, *J. Clin. Microbiol.* **25:**230–237.
433. Matthews, R., Wells, C., and Burnie, J. P., 1988, Characterization and cellular localization of the immunodominant 47-Kda antigen of *Candida albicans*, *J. Med. Microbiol.* **27:**227–232.
434. Manning-Zweerink, M., Maloney, C. S., Mitchell, T. G., and Weston, H., 1986, Immunoblot analyses of *Candida albicans*-associated antigens and antibodies in human sera, *J. Clin. Microbiol.* **23:**46–52.
435. Reen, D. J., 1988, Assessment of immunoblotting in the investigation of specific immune responses during infection, *Biochem. Soc. Trans.* **16:**132–134.
436. Weller, B. I., Simmons, P. D., Ivanyl, L., 1990, Identification of immunodominant antigens of *Candida albicans* in patients with superficial candidosis, *Clin. Immunol. Immunopathol.* **54:** 347–353.
437. Greenfield, R. A., Bussey, M. J., Stephens, J. L., and Jones, J. M., 1983, Serial enzyme-linked immunosorbent assays for antibody to *Candida* antigens during induction chemotherapy for acute leukemia, *J. Infect. Dis.* **148:**275–283.
438. Strockbine, N. A., Largen, M. T., and Buckley, H. R., 1984, Production and characterization of three monoclonal antibodies to *Candida albicans* proteins, *Infect. Immun.* **43:**1012–1018.
439. Ho, Y. M., Ng, M. H., Teoh-Chan, C. H., Yue, P. C. K., and Huang, C. T., 1976, Indirect immunofluorescence assay for antibody to germ tube of *Candida albicans*—a new diagnostic test, *J. Clin. Pathol.* **29:**1007–1010.
440. Quindos, G., Ponton, J., and Cisterna, R., 1987, Detection of antibodies to *Candida albicans* germ tube in the diagnosis of systemic candidiasis, *Eur. J. Clin. Microbiol.* **6:**142–146.
441. Quindos, G., Ponton, J., Cisterna, R., and Mackenzie, D. W. R., 1990, Value of detection of antibodies to *Candida albicans* germ tube in the diagnosis of systemic candidosis, *Eur. J. Clin. Microbiol. Infect. Dis.* **9:**178–183.
442. McHugh, T. M., Wang, Y. J., Chong, H. O., Blackwood, L. L., and Stites, D. P., 1989, Development of a microsphere-based fluorescent immunoassay and its comparison to an enzyme immunoassay for the detection of antibodies to three antigen preparations from *Candida albicans*, *J. Immunol. Methods* **116:**213–219.
443. Weiner, M. H., and Yount, W. J., 1976, Mannan antigenemia in the diagnosis of invasive *Candida* infections, *J. Clin. Invest.* **58:**1045–1053.
444. Kerkering, T. M., Espinel-Ingroff, A., and Shadomy, S., 1979, Detection of *Candida* antigenemia by counterimmunoelectrophoresis in patients with invasive candidiasis, *J. Infect. Dis.* **140:**659–664.
445. Weiner, M. H., and Coats-Stephen, M., 1979, Immunodiagnosis of systemic candidiasis: mannan antigenemia detected by radioimmunoassay in experimental and human infections, *J. Infect. Dis.* **140:**989–993.

446. Segal, E., Berg, R. A., Pizzo, P. A., and Bennett, J. E., 1979, Detection of *Candida* antigen in sera of patients with candidiasis by an enzyme-linked immunosorbent assay-inhibition technique, *J. Clin. Microbiol.* **10:**116–118.
447. Reiss, E., Stockman, L., Kuykendall, R. J., and Smith, S. J., 1981, Dissociation of mannan-serum complexes and detection of *Candida albicans* mannan by enzyme immunoassay variations, *Clin. Chem.* **28:**306–310.
448. Lew, M. A., Siber, G. R., Donahue, D. M., and Maiorca, F., 1982, Enhanced detection with an enzyme-linked immunosorbent assay of *Candida* mannan in antibody-containing serum after heat extraction, *J. Infect. Dis.* **145:**45–56.
449. Lemieux, C., St-Germain, G., Vincelette, J., Kaufman, L., and deRepentigny, L., 1990, Collaborative evaluation of antigen detection by a commercial latex agglutination test and enzyme immunoassay in the diagnosis of invasive candidiasis, *J. Clin. Microbiol.* **28:** 249–253.
450. Kahn, F. W., and Jones, J. M., 1986, Latex agglutination test for detection of *Candida* antigens in sera of patients with invasive candidiasis, *J. Infect. Dis.* **153:**579–585.
451. deRepentigny, L., Marr, L. D., Keller, J. W., Carter, A. W., Kuykendall, R. J., Kaufman, L., and Reiss, E., 1985, Comparison of enzyme immunoassay and gas–liquid chromatography for the rapid diagnosis of invasive candidiasis in cancer patients, *J. Clin. Microbiol.* **21:** 972–979.
452. Phillips, P., Dowd, A., Jewesson, P., Radigan, G., Tweeddale, M. G., Clarke, A., Geere, I., and Kelly, M., 1990, Nonvalue of antigen detection immunoassays for diagnosis of candidemia, *J. Clin. Microbiol.* **28:**2320–2326.
453. Fung, J. C., Donta, S. T., and Tilton, R. C., 1986, *Candida* detection system (CAND-TEC) to differentiate between colonization and disease, *J. Clin. Microbiol.* **24:**542–547.
454. Fujita, S., Matsubara, F., and Matsuda, T., 1986, Enzyme-linked immunosorbent assay measurement of fluctuations in antibody titer and antigenemia in cancer patients with and without candidiasis, *J. Clin. Microbiol.* **23:**568–575.
455. Gentry, L. O., Wildinson, I. D., Lea, S. S., and Price, M. F., 1983, Latex agglutination test for detection of *Candida* antigen in patients with disseminated disease, *Eur. J. Clin. Microbiol.* **2:** 122–128.
456. Piens, M.-A., Guyotat, D., Archimbaud, E., Plotton, C., Maupas, J., Mojon, M., and Fiere, D., 1988, Evaluation of a *Candida* antigen detection test (Cand-TecTM) in the diagnosis of deep candidiasis in neutropenic patients, *Eur. J. Cancer Clin. Oncol.* **24:**1655–1659.
457. Ness, M. J., Vaughan, W. P., and Woods, G. L., 1989, *Candida* antigen latex test for detection of invasive candidiasis in immunocompromised patients, *J. Infect. Dis.* **159:**495–502.
458. Escuro, R. S., Jacobs, M., Gerson, S. L., Machicao, A. R., and Lazarus, H. M., 1989, Prospective evaluation of a *Candida* antigen detection test for invasive candidiasis in immunocompromised adult patients with cancer, *Am. J. Med.* **87:**621–627.
459. Price, M. F., and Gentry, L. O., 1990, *Candida* antigen latex test, *J. Infect. Dis.* **161:**807.
460. Price, M. F., and Gentry, L. O., 1986, Incidence and significance of *Candida* antigen in low-risk and high-risk patient populations, *Eur. J. Clin. Microbiol.* **5:**416–419.
461. Bailey, J. W., Sada, E., Brass, C., and Bennett, J. E., 1985, Diagnosis of systemic candidiasis by latex agglutination for serum antigen, *J. Clin. Microbiol.* **21:**749–752.
462. Ness, M. J., Rennard, S. I., Vaughn, W. P., Ghafouri, M. A., and Linder, J. A., 1988, Detection of *Candida* antigen in bronchoalveolar lavage fluid, *Acta Cytol.* **32:**347–352.
463. Araj, G. F., Hopfer, R. L., Chesnut, S., Fainstein, V., and Bodey, G. P., 1982, Diagnostic value of the enzyme-linked immunosorbent assay for detection of *Candida albicans* cytoplasmic antigen in sera of cancer patients, *J. Clin. Microbiol.* **16:**46–52.
464. Burnie, J., 1985, A reverse passive latex agglutination test for the diagnosis of systemic candidosis, *J. Immunol. Methods* **82:**267–280.
465. Ferraira, R. P., Yu, B., Niki, Y., and Armstrong, D., 1990, detection of *Candida* antigenuria in disseminated candidiasis by immunoblotting, *J. Clin. Microbiol.* **28:**1075–1078.

466. Longbottom, J. J., Murray, I. G., and Pepys, J., 1963, Diagnosis of fungal disease, in: *Clinical Aspects of Immunology*, (P. L. Gell and R. R. A. Coombs, eds.), Blackwell Scientific Publications, Oxford, p. 71.
467. Shannon, D. C., Gohnson, G., Rosen, F. S., and Austen, K. F., 1966, Cellular reactivity to *Candida albicans* antigen, *N. Engl. J. Med.* **275:**690–693.
468. Hassett, A. M., Woods, R. J., Temperley, I. J., and Mullins, G. M., 1977, Cell mediated immunity to recall antigens in vivo and in vitro, *Irish J. Med. Sci.* **146:**167–174.
469. Ferguson, A. C., Kershnar, H. E., Collin, W. K., and Stiehm, E. R., 1977, Correlation of cutaneous hypersensitivity with lymphocyte response to *Candida albicans*, *Am. J. Clin. Pathol.* **68:**499–540.
470. Hogan, T. F., Borden, E. C., Freeberg, B. L., Vieau, J., Hsieh, F.-Y., and Crowley, J., 1980, Enhancement in recall antigen responses by frequent, repetitive skin testing with *Candida*, mumps, and streptokinase-streptodornase in 38 normal adults, *Cancer Immunol. Immunother.* **10:**27–31.
471. Valdimarsson, H., Higgs, J. M., Wells, R. S., Yamamura, M., Hobbs, J. R., and Holt, P. J. L., 1973, Immune abnormalities associated with chronic mucocutaneous candidiasis, *Cellular Immunol.* **6:**348–361.
472. Bice, D. E., Lopez, M., Rothschild, H., and Salvaggio, J., 1974, Comparison of *Candida*-delayed hypersensitivity skin test size with lymphocyte transformation, migration inhibitory factor production and antibody titer, *Int. Arch. Allergy* **47:**54–62.
473. Alford, R. H., 1973, Transformation of lymphocytes of normal and hospitalized adults by *Candida albicans* extract, *Proc. Soc. Exp. Biol. Med.* **144:**826–829.
474. Foroozanfar, N., Yamamura, M., and Hobbs, J. R., 1974, Standardization of lymphocyte transformation to *Candida* immunogen, *Clin. Exp. Immunol.* **16:**301–310.
475. Esch, R. E., and Buckley, C. E., 1988, A novel *Candida albicans* skin test antigen: efficacy and safety in man, *J. Biol. Standardization* **16:**33–43.
476. Hurtrel, B., and Lagrange, P. H., 1978, Delayed type hypersensitivity reactions to *Candida albicans* in mice, *Ann. D'Immunol.* **C129:**653–668.
477. Ponton, J., Quindos, G., Regulez, P., and Cisterna, R., 1985, Cellular and humoral immune responses to *Candida-albicans* in subcutaneously infected mice, *Mycopathologia* **92:**11–18.
478. Suzuki, M., and Hayashi, Y., 1975, Skin reaction and macrophage migration inhibition tests for polysaccharides from *Aspergillus fumigatus* and *Candida albicans*, *Jap. J. Microbiol.* **19:** 355–362.
479. Reiss, E., Stone, S. H., and Hasenclever, H. R., 1974, Serologic and cellular immune activity of peptidoglucomannan fractions of *Candida albicans* cell walls, *Infect. Immun.* **9:**881–890.
480. Ellsworth, J. H., Reiss, E., Bradley, R. L., Chmel, H., and Armstrong, D., 1977, Comparative serological and cutaneous reactivity of candidal cytoplasmic proteins and mannans separated by affinity for concanavalin A., *J. Clin. Microbiol.* **5:**91–99.
481. Domer, J. E., and Moser, S. A., 1978, Experimental murine candidiasis: cell-mediated immunity after cutaneous challenge, *Infect. Immun.* **20:**88–98.
482. Moser, S. A., Domer, J. E., and Mather, F. J., 1980, Experimental murine candidiasis: cell mediated immunity after cutaneous challenge, *Infect. Immun.* **27:**140–149.
483. Levy, R., Segal, E., Eylan, E., and Barr-Nea, L., 1983, Cell-mediated immunity following experimental vaccinations with *Candida albicans* ribosomes, *Mycopathologia* **83:**161–168.
484. Crowle, A. J., 1975, Delayed hypersensitivity in the mouse, *Adv. Immunol.* **20:**197–264.
485. Dikeacou, R., and Drouhet, E., 1981, Cellular inflammatory response to fungal antigen studied with the skin window technique—correlation of the exudate with the evolution of the mycotic infection, *Agents Actions* **11:**631–633.
486. Winterrowd, G. E., and Cutler, J. E., 1983, *Candida albicans*-induced agglutinin and immunoglobulin E responses in mice, *Infect. Immun.* **41:**33–38.
487. Domer, J. E., 1989, *Candida* cell wall mannan: a polysaccharide with diverse immunologic properties, *CRC Crit. Rev. Microbiol.* **17:**33–51.

488. Ausiello, C. M., Spagnoli, G. C., Boccanera, M., Casalinuovo, I., Malavasi, F., Casciani, C. U., and Cassone, A., 1986, Proliferation of human peripheral blood mononuclear cells induced by *Candida albicans* and its cell wall fractions, *J. Med. Microbiol.* **22:**195–202.
489. Piccolella, E., Lombardi, G., and Morelli, R., 1980, Human lymphocyte-activating properties of a purified polysaccharide from *Candida albicans*: B and T cell cooperation in the mitogenic response, *J. Immunol.* **125:**2082–2088.
490. Gettner, S. M. M., and Mackenzie, D. W. R., 1981, Responses of human peripheral lymphocytes to soluble and insoluble antigens of *Candida albicans*, *J. Med. Microbiol.* **14:** 333–340.
491. Tollemar, J., Ringden, O., and Holmberg, K., 1989, *Candida albicans*: mannan and protein activation of cells from various human lymphoid organs, *Scand. J. Immunol.* **30:**473–480.
492. Kerridge, D., and Whelan, W. L., 1984, The polyene macrolide antibiotics and 5-fluorocytosine Molecular actions and interactions, in: *Mode of Action of Antifungal Agents*, (A. P. J. Trinci, and J. F. Riley, eds.), London, British Mycological Society, pp. 343–375.
493. Bennett, J. E., 1990, Antifungal agents, in: *Principles and Practice of Infectious Diseases*, third edition, (G. L. Mandell, R. G. Douglas, and J. E. Bennett, eds.), Churchill Livingstone, New York, pp. 361–370.
494. Smego, R. A., Jr., Perfect, J. R., Durack, D. T., 1984, Combined therapy with amphotericin B and 5-fluorocytosine for *Candida* meningitis, *Rev. Infect. Dis.* **6:**791–801.
495. Diasio, R. B., Bennett, J. E., and Myers, C. E., 1978, Mode of action of 5-fluorocytosine, *Biochem. Pharmacol.* **27:**703–707.
496. Polak, A., and Scholer, H. J., 1975, Mode of action of 5-fluorocytosine and mechanisms of resistance, *Chemother.* **21:**113–130.
497. Vanden Bossche, H., Bellens, D., Cools, W., Gorrens, J., Marichal, P., Verhoeven, H., Willemsens, G., De Coster, R., Beerens, D., Haelterman, C., Coene, M.-C., Lauwers, W., and Le Jeune, L., 1986, Cytochrome P-450: target for itraconazole, *Drug Dev. Res.* **8:**287–298.
498. Dismukes, W. E., 1988, Azole antifungal drugs: old and new, *Ann. Intern. Med.* **109:** 177–179.
499. Meunier, F., Gerain, J., Snoeck, R., Libotte, F., Lambert, C., Cueppers, A. M., 1987, Fluconazole therapy of oropharyngeal candidiasis in cancer patients, in: *Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents*, (R. A. Fromtling, ed.), J. R. Prouss, Barcelona, pp. 169–174.
500. Dupont, B., and Drouhet, E., 1988, Fluconazole in the management of oropharyngeal candidosis in a predominantly HIV antibody-positive group of patients, *J. Med. Vet. Mycol.* **26:**67–71.
501. Meunier-Carpentier, F., Cruciani, M., Klastersky, J., 1983, Oral prophylaxis with miconazole or ketoconazole of invasive fungal disease in neutropenic cancer patients, *Eur. J. Cancer Clin. Oncol.* **19:**43–48.
502. Jones, P. G., Kauffmann, C. A., McAuliffe, L. S., Liepman, M. K., and Bergman, A. G., 1984, Efficacy of ketoconazole versus nystatin in prevention of fungal infections in neutropenic patients, *Arch. Intern. Med.* **144:**549–551.
503. Meunier, F., Leleux, A., Snoeck, R., Gerain, J., Lambert, C,. Cueppers, A. M., 1989, Chemoprophylaxis of fungal infections, in: *Diagnosis and Therapy of Systemic Fungal Infections*, (K. Holmberg, and R. Meyer, eds.), Raven Press, New York, pp. 167–177.
504. Edwards, J. E., Jr., 1990, *Candida* species, in: *Principles and Practice of Infectious Diseases*, (G. L. Mandell, R. G. Douglas, and J. E. Bennett, eds.), Churchill-Livingstone, New York, pp. 1943–1958.
505. Meunier, F., 1989, Candidiasis, *Eur. J. Clin. Microbiol. Infect. Dis.* **8:**438–447.