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Production and Function of Cytokines in Natural and Acquired Immunity to *Candida albicans* Infection

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INTRODUCTION

The yeast *Candida albicans* is a common commensal organism in humans and animals, and it is a major opportunistic fungal pathogen (244). Mucocutaneous infections—commonly known as thrush—are the usual manifestation of the disease, and although not normally life-threatening, they represent a problem of considerable socioeconomic importance. Oral candidiasis is frequently seen in dental practice (163) and in patients with human immunodeficiency virus infection or AIDS (185), and *Candida* vaginitis is one of the more common ailments treated by general practitioners (113).

Severe, intractable mucocutaneous infections are less com-

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TABLE 1. Summary of the cytokines relevant to *C. albicans* infection and their major functions

Cytokine	Major functions[a]
IL-1	Mediates acute-phase response; acts with other cytokines to activate T- and B cells; induces hematopoietic growth factors
IL-2	Induces clonal expansion of and cytokine synthesis by antigen-specific T cells
IL-3	Stimulates proliferation and differentiation of hematopoietic stem cells
IL-4	Modulates T- and B-cell differentiation, growth, and function; induces NK and LAK cell activity; enhances macrophage antigen processing and presentation
IL-6	Induces acute-phase proteins; stimulates T-cell differentiation, growth, and effector function
IL-8	Mediates neutrophil chemotaxis; exerts other proinflammatory effects
IL-10	Inhibits cytokine secretion by T cells and monocytes; stimulates B-cell proliferation and differentiation
IL-12	Stimulates growth and IFN-γ production by T cells; enhances NK and LAK activity
IFN-γ	Activates macrophages, PMNL, and NK cells; increases MHC class II antigen expression; inhibits virus growth and cell proliferation
TNF-α	Affects growth and differentiation of T cells; induces adhesion molecules; activates endothelial cells
TGF-β	Suppresses T- and B-lymphocyte functions; stimulates inflammatory cells
G-CSF	Stimulates proliferation and differentiation of neutrophils; enhances activity and survival of the mature cells
GM-CSF	Induces growth and differentiation of granulocyte/macrophage progenitor cells; enhances the function of the mature cells
M-CSF (CSF-1)	Stimulates the growth of precursor cells and enhances the function of mature macrophages

[a] The function and regulation of these cytokines have been discussed in detail in recent reviews (6, 188).

mon and fall into two broad categories, chronic mucocutaneous candidiasis and chronic vaginal candidiasis. These are discussed separately below. Systemic infections occur in patients whose bodily defenses have been compromised by cancer, by major surgery, or by treatment with cytotoxic or immunosuppressive drugs. Indeed, the increasing sophistication of medical technology has unfortunately been associated with an upsurge in *Candida* infection in the hospital environment (258), to the extent that it now causes 7% of all nosocomial infections (347) and is directly responsible for morbidity and mortality substantially in excess of that attributable to the patients' underlying conditions (355).

Oral and vaginal thrush can be triggered by a variety of changes in physiological and immunological homeostasis (62, 313). Chronic mucocutaneous candidiasis and other severe mucocutaneous infections are usually associated with defects or weaknesses in the cell-mediated immune response (182), whereas hematogenous and disseminated infections are more common in patients with dysfunctional neutrophils (181) or neutropenia (57, 195). These observations suggest that both phagocytic cells and specific cell-mediated immune responses participate in the host response against the organism; but despite the fact that many different modes of *Candida* killing have been demonstrated in in vitro systems, those that function in vivo have not yet been convincingly defined.

Evidence is accumulating that T cells contribute to the host response, in both mucocutaneous and experimental systemic infections, by providing cytokine-mediated activation signals to mononuclear and polymorphonuclear phagocytes (Table 1). In developing a synthesis of these data, however, two crucial themes must be borne in mind. The first is whether fundamentally different mechanisms are responsible for recovery from mucosal as distinct from systemic infections, and the second relates to the specific changes or abnormalities in the host response that predispose to recurrent infection.

Overview

The mechanisms of *Candida* killing and their regulation by cytokines have been subject to detailed analysis in vitro with both human and mouse phagocytic cells. However, in vivo, there is a crucial difference between animal models of candidiasis and the human disease in that most humans are colonized with *C. albicans* shortly after birth and, in adult life, infections occur in the presence of both humoral and cell-mediated immune responses to antigens of the yeast. Hence, a true "primary" infection in humans occurs only in the early neonate, and it is necessary to exercise some caution in applying the results of studies in humans to an understanding of the underlying mechanisms of host resistance.

It is difficult to make direct comparisons between human and animal studies, and our knowledge has not yet advanced to the point where it is possible to draw general conclusions. For this reason, results from the two experimental systems are considered separately. The different manifestations of human infections with *C. albicans* are described, and this is followed by an outline of cellular responses to the yeast. The utility of animal models is then addressed, with particular emphasis on strain variation and the influence of genetic factors on pathogenesis and the evolution of the immune response. With this background, models of mucosal and systemic infection are discussed, and we conclude by considering immunoregulatory interactions in *Candida* infection and their role in the establishment of chronic disease.

HUMAN STUDIES

Chronic Mucocutaneous Candidiasis

Chronic mucocutaneous candidiasis is an all-encompassing term for a collection of syndromes characterized by recurrent infection of the mucous membranes, skin, and nails by *C. albicans*. Although the disease is frequently seen in association with endocrinopathies, it is generally recognized that the main predisposing factor is a defect in the cell-mediated immune response (183, 184). Patients with chronic mucocutaneous candidiasis show a number of different patterns of abnormalities in immunological responsiveness (Table 2), but it is important to note that there is no *Candida*-specific defect common to all groups. Nevertheless, therapy directed at restoration of cellular immune function generally results in remission, and this link between systemic cell-mediated immunity and mucosal defense mechanisms has been crucial in developing an understanding of the pathogenesis of the disease.

Chronic Vaginal Candidiasis

It is estimated that three out of four women will experience at least one episode of vaginal candidiasis during their reproductive years (161), and the prevalence of *Candida* carriage in the vagina ranges from 20% in New Zealand (5) to 40% in

TABLE 2. Patterns of abnormalities of cellular immunity in patients with chronic mucocutaneous candidiasis[a]

Group	DTH (*Candida*)	No. of T cells	Lymphocyte transformation		MIF production	
			Candida	Mitogen	*Candida*	Mitogen
1	Anergy	Few	SN	SN	SN	SN
2	Anergy	N	SN	N	SN	N
3	Anergy	N	N	N	SN	N
4	Negative	N	SN	N	SN	N
5	Negative	N	N	N	SN	N
6	Negative	N	SN	N	N	?
7	Negative	N	N	N	N	N

[a] Reproduced with permission from Kirkpatrick (184). Abbreviations: MIF, migration inhibitory factor; SN, subnormal or absent; N, normal response; ?, value unknown.

Nigeria (118). Although some women never contract vulvovaginal candidiasis and others experience infrequent episodes, a third subpopulation, possibly comprising as many as 5% of all adult women (314), suffer from recurring infections that result in morbidity and physical and emotional distress. Numerous environmental and lifestyle factors (pregnancy, oral contraceptives, antibiotics, diabetes mellitus, tight clothing, etc.) have been identified as risk factors for *Candida* vaginitis, but the majority of women with recurrent vulvovaginal candidiasis do not have recognizable predisposing factors (313).

Immune defects in chronic vaginitis. Recurrent vaginal candidiasis occurs in ostensibly immunocompetent women who are not unusually susceptible to other infections, in the presence of established humoral and cell-mediated immune responses against the yeast. A comparison of antibody profiles from women with chronic recurrences and appropriate controls has not revealed any consistent differences in either serum (162) or vaginal secretions (142), although one study identified a unique band pattern in cervicovaginal secretions from culture-positive patients with a classical clinical presentation (306). Nevertheless, it seems probable that susceptibility in these women is associated with some form of T-lymphocyte defect.

One of the major difficulties in approaching a systematic analysis of immune deficiencies in *Candida* vaginitis is the variability in severity of symptoms of the disease (160). As is the case in chronic mucocutaneous candidiasis, chronic vaginal candidiasis may be the outward expression of a number of different immunological defects. For example, *Candida* vaginitis is associated with both allergic hypo- and hyperreactivity (219), as well as with polymorphisms in the third component of complement (18). Superimposed on these factors are (i) variations in T-lymphocyte physiology during the menstrual cycle (264), (ii) changes in nonspecific (209) and specific (174) immune responses related to fluctuations in the levels of progesterone or estradiol, (iii) the effects of genetically determined or progesterone-mediated alterations in monocyte accessory cell function (175), and (iv) the influence of sex steroids, such as progesterone, on the yeast-mycelium transition and the candidacidal activity of neutrophils (239).

Nonetheless, studies of acquired immunity in women with chronic *Candida* vaginitis have found evidence of deficiencies in cell-mediated immune responsiveness (326). The proliferative response to stimulation with *Candida* antigens of lymphocytes from patients with vaginal candidiasis was found to be specifically reduced in comparison with controls (361), and the response of the control lymphocytes could be blocked by coculture with either serum or lymphocytes from the patients. *Candida*-specific antibody decreased lymphocyte proliferation by inhibiting the uptake and/or processing of *Candida* antigens by macrophages and the recognition by lymphocytes of *Candida* antigen on the macrophage surface (358). When the effect of macrophages on *Candida*-specific lymphoproliferation was assessed directly in cell-mixing experiments (359), the response of lymphocytes from the patients was restored to the normal range if they were incubated in the presence of control macrophages. Conversely, macrophages from the patients inhibited the responses of control lymphocytes. Inhibition could be reversed by the addition to the cultures of ibuprofen or indomethacin, showing that the effect was due to the secretion by macrophages of prostaglandin E_2, which blocked lymphocyte proliferation.

Different patterns of cell-mediated immune responses have been reported in other studies. One series of patients with recurrent vaginal candidiasis showed low T-lymphocyte counts twice as frequently as controls (223), and in this group, in vitro proliferation to *Candida* antigens bore no relation to the course of the disease. A more comprehensive longitudinal study demonstrated a transient loss of *Candida*-specific delayed cutaneous skin test reactivity during episodes of symptomatic vaginitis (124), but *Candida*-specific lymphocyte proliferation and lymphokine production were similar to those in controls during both acute episodes of vaginitis and periods of remission.

In some patients with *Candida* vaginitis, antifungal treatment does not invariably alleviate symptoms. *Candida*-specific immunoglobulin E (IgE) antibodies as well as prostaglandin E_2 were identified in vaginal fluid from a high proportion of subjects in this category (360), suggesting that a vaginal allergic response might induce prostaglandin E_2 synthesis, thus suppressing cell-mediated immune responses and predisposing to recurrent infection. In a subsequent clinical trial (272), there was a significant reduction in the frequency of recurrences after desensitization in a group of patients unresponsive to all other forms of therapy.

Given the number and complexity of the factors that can influence clinical presentation, it is hardly surprising that there is little agreement among the various studies of immune function in these patients. It is important to recognize that even in carefully matched patients with culture-proven infections, antigen processing and host responses may vary. This is exemplified by the detection of antibodies to enolase, a protein that acts as an immunodominant antigen in both humans (131, 349) and mice (325), in only a proportion of a cohort of patients with *Candida* vaginitis, grouped according to the presence or absence of active infection (247).

Many different mucosal models have been studied in attempts to identify the responses that might be deficient or abnormal in these women, with little success; and it is clear that progress in this area will depend on a more complete understanding of the complex immunological relationships between the yeast and the host.

CELLULAR RESPONSES TO *CANDIDA* INFECTION

Lymphocyte Proliferative Responses

In most cases of chronic mucocutaneous candidiasis and chronic vaginal candidiasis, *Candida*-specific lymphoproliferation is impaired. Considerable effort has been directed towards an analysis of this response, in terms of the requirements for stimulation, cytokine production by the responding lymphocytes, and, more recently, identification of the protein recognized by the T cells.

Human lymphocytes show strong proliferative responses after stimulation in vitro with antigens of *C. albicans*, and this is dependent upon a degree of genetic compatibility between antigen-presenting cells and the responding lymphocytes. Activation of T lymphocytes by *Candida* allergen was found to require the presence of human leukocyte antigen (HLA)-Dw1.DR1- or Dw12.DR2-compatible macrophages (241), and the magnitude of the proliferative response showed a good correlation with the HLA type of the responding cells (240). In particular, a significant association was found between low responses and HLA-B15, whereas high responses correlated with HLA-B7 as well as Dw1.

Lymphoproliferation could be induced by culture either with glutaraldehyde-inactivated *C. albicans* blastospores or with a phosphorylated glucomannan-protein complex of the cell wall (32), although a low-protein mannan extract was much less active. After 7 to 10 days of incubation, the cultured cells displayed some but not all natural killer (NK) cell markers, expressed cytotoxicity against both NK-susceptible and NK-resistant tumor cell lines, and produced both interleukin-2 (IL-2) and gamma interferon (IFN-γ) (31). A monoclonal antibody against the class II determinant of the HLA complex inhibited proliferation irrespective of the *Candida* antigen used (32), again indicating that presentation took place through major histocompatibility complex (MHC) class II molecules.

Stimulation of human peripheral blood mononuclear cells (PBMC) with *C. albicans* or its antigens also results in the production of a number of different cytokines. PBMC from healthy female donors produced both tumor necrosis factor (TNF) and IL-1 in proportion to the concentration of viable organisms used for challenge (167), although the levels of IL-1 and TNF produced by any individual appeared to be independently regulated. Differences in the time course of cytokine production were also observed (151), in that IFN-γ was produced on day 1 after stimulation, whereas synthesis of TNF-α occurred more slowly. Analysis of the expression of cytokine genes in cultures of human PBMC stimulated either with *Candida* antigen (139) or with mannoprotein constituents of *C. albicans* (33) revealed early and long-lasting production of mRNAs for IL-1β, TNF-α, and IL-6, as well as appreciable levels of mRNAs for IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IFN-γ. When cytokine protein synthesis was inhibited by cycloheximide, a superinduction of mRNAs for IL-4, IL-10, and, more markedly, IFN-γ was observed (33). Mannoprotein was unable to stimulate consistent expression of the genes encoding IL-4, IL-5, or IL-10.

Separation of the crude, carbohydrate-rich extract of the *C. albicans* cell wall into two major proteoglycan constituents revealed that immunogenic activity was associated with a high-molecular-mass, polydisperse material (333). This fraction was as efficient as the crude extract in inducing lymphocyte proliferation, production of IL-2 and IFN-γ, and generation of cytotoxicity against NK-sensitive target cells. The active constituent was not mitogenic but acted through interaction with the T-cell receptor complex. Chromatographic separation identified a 65-kDa mannoprotein that stimulated proliferation and production of IL-1β, IFN-γ, and IL-6 but not IL-4 from human PBMC as the immunodominant antigen for cell-mediated immune responses in normal humans (332). When administered to mice, this preparation elicited strong delayed-type hypersensitivity (DTH) but relatively poor protective responses (222).

In addition to the stimulatory activities that have already been defined, members of a family of mannose oligosaccharides derived from a cetyltrimethylammonium bromide preparation of *C. albicans* cell wall were potent inhibitors of lymphoproliferation stimulated by *Candida* and other antigens (259). The properties and biological functions of mannan have been the subject of excellent reviews (109, 236), and the regulation of *Candida*-specific immune responses will be further considered below.

Candidacidal Activity of Phagocytic Cells

Polymorphonuclear leukocytes (PMNL) represent the first line of defense in the control of *Candida* infections. Optimal phagocytosis by human PMNL requires fresh human serum (254), the opsonic activity of which depends primarily on complement activated via both the alternative and classical pathways (316). Human serum deficient in either the second (224) or the fifth (295) component of complement was able to efficiently opsonize *Saccharomyces cerevisiae* or *C. albicans* for ingestion by human neutrophils, but in the absence of C3, phagocytosed yeasts were not killed (363). Activation of C3 also generates chemotactic factors (265) that act to recruit leukocytes to the site of infection.

In vitro, PMNL readily ingest large numbers of *Candida* yeasts (254), and a constant percentage of organisms are killed within the cell regardless of the number ingested. The remainder germinate (270) and grow out through the cell membrane. The relatively low efficiency of killing within human neutrophils may be associated with functional differences in the two classes of phagosomes (72), as incompletely sealed vacuoles support antimicrobial processes substantially less well than completely sealed ones. The yeast, too, may differ in susceptibility to intracellular killing. More blastospores of strains that were virulent for mice survived intracellularly in human and mouse PMNL than did those of attenuated strains (270). Furthermore, progeny from blastospores of the attenuated strain that had germinated within the PMNL regained virulence for mice and behaved similarly to the virulent strains in tests with human and mouse phagocytes in vitro.

In contrast, hyphal elements may be more susceptible to attack by PMNL (81). For example, human neutrophils are highly efficient in clearing germinating *Candida* cells from endothelial cell monolayers (115). Killing is mediated by activation of the respiratory burst and focal release of toxic oxygen species, but the generation of oxidants may not always be sufficient to mediate hyphal killing in the absence of complementary, nonoxidative mechanisms (319). In addition to direct killing of the yeasts or hyphae, death of PMNL in a lesion may also act to control the infection, as dying PMNL release a substance that has a strong candidastatic effect (218). This was identified as an abundant calcium-binding protein with generalized antimicrobial activity, originally described in neutrophils as the L1 myelomonocytic antigen or the cystic fibrosis antigen (315), and now termed calprotectin (320).

Nonspecific activation of PMNL by treatment with lipopolysaccharide, for example, strongly enhanced growth inhibition of *C. albicans* by increasing the number of yeasts ingested per neutrophil as well as the number of neutrophils phagocytosing fungal cells (248). Activation resulted in the production of IL-1, IL-6, and TNF-α, but the antifungal activity was shown to be mediated by the release of the iron-binding protein lactoferrin. Furthermore, a mannoprotein component of *C. albicans* was shown to be as efficient as lipopolysaccharide, GM-CSF, and IL-8 in potentiating lactoferrin-mediated inhibition of *Candida* growth in vitro (249).

Activation may also be mediated via specific receptors on the cell surface. Human PMNL constitutively express detectable levels of the β-chain but not the α-chain of the IL-2 receptor and respond functionally to IL-2 by enhanced lacto-

TABLE 3. Effect of cytokines on killing of *C. albicans* by human phagocytes

Cytokine	Cell type	Effect on *Candida* killing	Reference(s)
IL-1	PMNL	Increased	119
IL-3	Monocytes	Increased	120, 312, 350
IL-8	PMNL	Increased	105
IFN-γ	Macrophages	Increased	204, 206, 235
	PMNL	Increased	98, 323
TNF-α	PMNL	Increased	101, 102, 121, 269
	PMNL	Decreased	98
G-CSF	PMNL	Increased	362
GM-CSF	PMNL	Increased	119
	Monocytes	Increased	120, 312, 350
M-CSF	Monocytes	Increased	350

ferrin-mediated antifungal activity (104). Oxidative metabolism, as measured by superoxide anion production, was not involved in this response. IL-2 binds to *C. albicans* (334) and, when complexed in this manner, increases the lymphoproliferative response of normal human peripheral blood lymphocytes to the yeast. The significance of this phenomenon in vivo has yet to be established, but it is not unreasonable to suggest that it may increase the efficiency of presentation of this cytokine to the PMNL.

Human cells of the monocyte/macrophage lineage also represent an important component of the host defense system against *Candida* infection. Peripheral blood monocytes are able to damage hyphal and pseudohyphal elements of *C. albicans* without completely ingesting them (97). The dominant mechanism of killing involves the myeloperoxidase-dependent pathway, although monocytes from patients with myeloperoxidase deficiency were also able to damage hyphae. The killing of *C. albicans* blastospores by human monocytes (299) and human alveolar macrophages (317) has also been attributed to oxidative mechanisms. In contrast, peritoneal macrophages were able to kill very few ingested *Candida* organisms (256), so the "innate" effector activity of these cells may depend on the source from which they were derived.

The yeast, however, may be able to defend itself actively against the effector activity of monocytes/macrophages. After 1 h of incubation with *C. albicans* blastoconidia, a proportion of human peripheral blood monocytes that had phagocytosed the yeast had died, as indicated by staining with ethidium bromide, even though the cell membranes showed no sign of penetration by the fungi (93). The mechanism by which the monocytes are killed remains to be clarified, and the relevance of this subtle interaction to the process of colonization and invasion in vivo is not yet clear.

Although freshly isolated monocytes and macrophages clearly express intrinsic anti-*Candida* activity, numerous studies have demonstrated that expression of the full candidacidal function of both PMNL and monocytes/macrophages requires activation either by colony-stimulating factors (CSFs) or by T-lymphocyte-derived cytokines (Table 3).

Effect of Cytokines on Human Phagocytes

Polymorphonuclear leukocytes. Cytokines can be elaborated not only by antigen-specific T lymphocytes but also by NK cells and by the phagocytic cells themselves. In humans, NK activity is mediated by a population of large granular lymphocytes. These cells are unable to kill *Candida* organisms directly (367) but, when activated with antigens in vitro, release TNF-α (103), GM-CSF (48), and an as yet uncharacterized PMNL-activating factor (101), all of which stimulate neutrophil-mediated inhibition of fungal growth (101, 102, 121, 269). Other cytokines, such as the monocyte-derived neutrophil chemotactic factor IL-8, have also been found to augment PMNL function in vitro. IL-8 stimulated antifungal activity in PMNL but not in monocytes (105); in contrast to IFN-γ and TNF-α, it did not stimulate the production of superoxide in the cells, suggesting that the fungal killing might take place via oxygen-independent pathways.

After exposure to the yeast, PMNL themselves have been shown to produce TNF-α (106), so that activation can take place in an exponential autocrine loop. TNF is chemotactic for PMNL and monocytes (226), so autocrine production of this cytokine may also act to increase the recruitment of inflammatory cells to the site of infection. PMNL can be activated to produce TNF-α not only by *C. albicans* and GM-CSF but also by IL-2, via binding to the β-chain of the IL-2 receptor expressed on the cell surface (352). Treatment of the neutrophils with cycloheximide did not affect induction of TNF-α mRNA by IL-2 but blocked induction by GM-CSF. In contrast, TNF-α mRNA was superinduced in cycloheximide-treated neutrophils activated by exposure to *C. albicans*. Thus, it is evident that transcription of the TNF-α genes is responsive to different cytokines and activating factors and that these are independently regulated. It has been further suggested (100) that the complexity of the neutrophil response to cytokines indicates that they may be active participants in the afferent phase of the immune response rather than simply being end-stage effector cells.

IFN-γ acts both separately from (323) and synergistically with (102) TNF-α to augment the candidacidal activity of human PMNL in vitro, although another study (98) demonstrated that the two cytokines have markedly different effects on neutrophil function. In the last study (98), priming of neutrophils with either IFN-γ or TNF-α increased early superoxide generation; but IFN-γ augmented hyphal killing, whereas TNF-α reduced neutrophil fungicidal activity to nearly 40% less than that of unprimed control cells, even though it enhanced superoxide responses more dramatically than IFN-γ. Furthermore, IFN-γ added during priming failed to correct TNF-α-associated functional defects in neutrophil anti-*Candida* responses. Thus, cytokine-induced augmentation of the respiratory burst may not necessarily reflect enhancement of the candidacidal activity of the cells.

This conclusion has been reinforced by studies on the effect of granulocyte colony-stimulating factor (G-CSF) on the oxidative burst and microbicidal activity of neutrophils. These experiments showed that G-CSF enhanced superoxide production in response to both opsonized blastoconidia and pseudohyphae (279) and significantly increased bacterial phagocytosis and bactericidal activity. Although treatment of normal human PMNL with recombinant G-CSF in vitro increases *Candida* killing (362), the activated neutrophils have been shown to exert fungicidal activity against pseudohyphal elements of the yeast (278) but not against blastoconidia (280). Two other cytokines, IL-1 and GM-CSF, have also been reported to enhance phagocytosis by human neutrophils (119).

Macrophages. Human monocytes respond to *C. albicans* by increasing production of complement factors (155) and GM-CSF (154), and these functions, as well as the expression of complement receptor 3 (CD11b/CD18), are regulated by transforming growth factor beta (TGF-β) (156). Monocytes also produce both TNF-α and IL-6 after stimulation in vitro with heat-killed clinical isolates or agerminative mutants of *C. albicans* (262, 263). TNF-α could be induced, in a dose-dependent fashion, by stimulation of human monocytes with a phospho-

lipomannan antigen of the yeast (171), although the yield was significantly increased by prestimulation of the cells with IFN-γ. These responses by the monocytes themselves may be an important mechanism both for opsonization of the fungus and for the initiation of a local inflammatory reaction.

Opsonized *Candida* species are ingested by both monocytes and monocyte-derived macrophages, but unopsonized *Candida* organisms are phagocytosed only by the latter, primarily via binding to the mannose receptor (204, 205). IFN-γ, typically produced by activated T lymphocytes, is one of the major factors that augment the phagocytic and candidacidal activities of human macrophages (235), and treatment with IFN-γ increases the capacity of monocytes and monocyte-derived macrophages to ingest and kill both opsonized (206) and unopsonized (204) Candida yeasts. Monocyte-derived macrophages from cord blood were much less responsive than those from adults (205), a significant finding in view of the increased susceptibility of neonates to infection. Killing was associated with a *Candida*-stimulated respiratory burst and release of myeloperoxidase-dependent oxidants and beta-glucuronidase in monocytes but not in monocyte-derived macrophages, which lack myeloperoxidase (203). Therefore, although monocytes and macrophages function similarly in their interaction with *C. albicans*, they appear to use different oxygen reactive products for intracellular killing (330). Surprisingly, the increased candidacidal activity of IFN-γ-activated macrophages was associated with reduced expression of the macrophage mannose receptor, suggesting that this might reflect enhanced coupling of this receptor to microbicidal functions (206).

Human alveolar macrophages also present an efficient barrier to infection with *C. albicans* (346), and treatment in vitro with IFN-γ, IL-1α, or lipopolysaccharide but not IL-2 significantly augments *Candida* killing. The candidacidal but not the phagocytic activity of alveolar macrophages declined progressively in culture, although this effect was reversible by exposure to IFN-γ, IL-1α, or lipopolysaccharide. Treatment of alveolar macrophages with a calcium ionophore showed that the phagocytic and killing events were ion dependent (343) and that the enhancement of intracellular candidacidal activity correlated with an augmented anti-*Candida* activity of cation-activated proteases.

Incubation of human monocytes with recombinant human GM-CSF or IL-3 enhanced both production of superoxide anion and cytotoxic activity for *C. albicans* (120, 312, 350). Macrophage CSF (M-CSF) also augmented *Candida* killing, but to a lesser extent (350). Under different experimental conditions, monocytes cultured in medium supplemented with autologous serum and a combination of recombinant human IFN-γ and IL-3 formed multinucleated giant cells that produced twice as much superoxide anion per unit of cytoplasmic protein as controls (117) and killed substantially more of the ingested yeasts. A more detailed examination of monocyte candidacidal activity in vitro (350) found that monocytes aged in medium lost their spontaneous candidacidal activity. The cultured monocytes did not reacquire activity after addition of IFN-γ, and supplementation of the medium with IFN-γ failed to maintain this function. In contrast, monocytes maintained in GM-CSF or IL-3 exhibited a high level of candidacidal activity throughout the period of culture, and these cytokines were also able to restore the response of cells aged in culture. Thus, GM-CSF and IL-3 may potentiate the anti-*Candida* activity of human monocytes via a different pathway than IFN-γ.

In addition, monocyte function can be regulated by interactions with lymphokine-activated killer (LAK) cells, which are derived from large granular lymphocytes by culture with IL-2. Electron microscopy has shown that LAK cells make intimate contact with the yeast, particularly the fungal germ tubes (7), but do not themselves display any consistent candidacidal or candidastatic activity. Exposure of cultured monocytes to LAK cells substantially suppressed their ability to control fungal growth (354), and prior activation of the monocytes with GM-CSF or IL-3 rendered them even more susceptible to the inhibitory effect. Processing and presentation of *C. albicans* antigens, as well as expression of mRNA for IL-1α and IL-1β in response to antigenic stimulation, were also downregulated in monocytes cultured with GM-CSF or IL-3 after exposure to LAK cells (353). Although IFN-γ was unable to sustain the candidacidal function of cultured monocytes (350), it protected them from inhibition by the LAK cells (353, 354).

These human experiments have revealed that the candidacidal function of human phagocytes can be modulated in a variety of ways by treatment with different cytokines in vitro and provided evidence that the effector functions are subject to regulation by other lymphoid cells. However, the relevance of the different effector pathways to the various manifestations of the human disease remains obscure, and the efficiency of cytokine therapy in modulating the course and outcome of infection has still to be firmly established. For example, local administration of recombinant human G-CSF to neutropenic AIDS patients resulted in a transient increase in neutrophil counts that was associated with a significant enhancement of their fungicidal activity (345); but even though infusion of M-CSF increased monocyte migration and phagocytosis of *C. albicans* (180), clinical trials of M-CSF therapy for fungal infections in bone marrow transplant recipients have not yet provided definitive evidence for a beneficial effect (237).

MURINE MODELS OF CANDIDIASIS

The crucial factors involved in recovery from and resistance to the human disease are almost impossible to identify because of the genetic heterogeneity of the population and the diverse and often unpredictable effects of other confounding factors, such as lifestyle and concomitant infections. The only way in which these variables can be eliminated or identified and their effects evaluated is by the use of animal models. It is not the intention here to review the many different animal models of *Candida* infection, which have been discussed in detail elsewhere (4); instead, the main focus will be on recent immunological studies in the mouse.

Characteristics of the Infection

Systemic candidiasis in inbred mice closely resembles the human disease. Both brain and kidney involvement are common in disseminated *Candida* infections in humans (244, 252), and in mice also, these organs are a prime focus for infection (199, 251). As the kidney possesses a phagocytic system which is able to eliminate the organisms as efficiently as those of the liver and spleen (36), its unusual susceptibility in mice is somewhat surprising but may be related to the extremely aggressive challenges used in many of the experimental systems. When the number of *Candida* organisms used to establish infection is reduced, the most obvious tissue damage occurs in the brain (251).

An unusual feature of these lesions is that the brains, in CBA/CaH mice in particular, often exhibit large accumulations of growing yeasts in the absence of any detectable inflammatory response (251). Abscesses, consisting of mixed inflammatory cells together with yeast and hyphal forms of the organism, form gradually, and the infection eventually resolves. With increasing doses, mice of all strains begin to die; however, in

TABLE 4. Patterns of susceptibility to *C. albicans* infection of inbred strains of mice

Strain	*H-2* type	C5 allele	Mortality	Brain damage[a]	No. of colonies in kidney	Antibody isotype[b]	Immunodominant antigen(s)[b] (kDa)
A/J	*a*	0	High	Mild	High	?	?
AKR	*k*	0	Moderate	Severe	Low	IgG2a	87, 96, 138
BALB/c	*d*	1	Low	Mild	Low	IgG1	48
CBA/CaH	*k*	1	Moderate	Severe	Low	IgG2a	87, 96, 138
DBA/1	*q*	1	Low	Mild	Low	?	?
DBA/2	*d*	0	High	Mild	High	IgG1	48

[a] Tissue damage was evaluated on the basis of the size and severity of abscesses in the brain, which is the organ that is most susceptible to infection and in which the lesions are most obvious.

[b] Data extracted from Constantino et al. (84).

our experience, deaths typically occur within the first 5 to 7 days (23), after which time survivors suffer no untoward effects except for a behavioral abnormality associated with chronic osteomyelitis in the vicinity of the inner ear (25). The proximal cause of death has not been unequivocally established, but as the kidneys of moribund mice that are killed for ethical reasons are distended with *Candida* organisms and appear grossly and microscopically abnormal, a likely reason for this early mortality is kidney failure caused by acute fungal pyelonephritis.

Infection in Inbred Strains

Insight into the basic mechanisms of host defense can best be obtained by comparing inflammatory and immune responses in inbred strains of mice that exhibit different patterns of susceptibility to infection with the yeast (Table 4). Susceptibility to systemic candidiasis has been evaluated by gross measures, such as mortality; by colony counts in infected tissues; and by histological evaluation of the severity of lesions in the tissues.

Mortality is a good correlate of susceptibility and resistance in viral and bacterial infections; however, in *Candida* infections, other factors appear to supervene. Opsonization by complement is critical in reducing the initial fungal burden, and when inbred strains are ranked by mortality or colony counts in the kidney, those deficient in the fifth component of complement die after challenge with substantially lower doses of *C. albicans* than complement-sufficient mice (16, 149). However, in complement-sufficient strains, there is no correlation between mortality or colonization patterns and other known genetic markers, such as those within the mouse MHC (207, 208).

Histopathological assessment has shown two distinct patterns of tissue destruction in the brains of inbred mice of different strains (21). These can be classified as severe, or type 1, lesions, characterized by numerous large abscesses containing yeast and mycelial growth forms within the necrotic debris together with an inflammatory infiltrate consisting of mononuclear and PMNL, and mild, or type II, lesions, which show similar characteristics but are small and infrequent. Mild and severe tissue damage can also be discerned in other organs, but to a lesser extent. However, the type I and type II patterns of tissue destruction do not correlate with either mortality or the number of viable organisms in the brain (16). Although the latter was generally greater in type 1 (CBA/CaH and AKR) than in type II (BALB/c and DBA/1) mice, the kinetics of fungal growth and clearance in the two types of mice were similar (15, 17), peaking on day 4 after infection and falling away rapidly thereafter. The propensity to develop mild or severe tissue damage is heritable, and studies in F_1 and F_2 hybrid mice have shown that this is controlled by a single codominant gene (or gene complex) that segregates in Mendelian fashion (27). The variables that determine the expression of type I or type II lesions have not yet been defined but may be related to either a qualitative or quantitative difference in the effector functions of bone marrow-derived phagocytic cells or a difference in the development or evolution of cell-mediated immune responses.

The expression of protective responses after infection also correlates with the patterns of tissue damage in type I and type II mice. When mice are infected with a sublethal dose of *C. albicans*, allowed to recover, and rechallenged, brain lesions in CBA/CaH (type I) mice are much smaller and less numerous than those in controls, whereas in BALB/c (type II) mice, the protective effect is less apparent (22). Protection can be passively transferred by serum from immune mice (28), suggesting that it is mediated by a *Candida*-specific antibody. Infection with *C. albicans* results in the production of a wide spectrum of antibodies, and studies in outbred mice have demonstrated protection mediated by an autoantibody against a 90-kDa heat shock protein (214). However, both the immunodominant epitopes recognized and the major antibody isotypes produced differ in type I and type II mice (Table 4).

Role of complement. As noted above, mice genetically deficient in the fifth component of complement are extremely susceptible to lethal challenge with *C. albicans* (149, 228), but its influence in determining patterns of resistance to infection in inbred strains has only recently been elucidated (17).

The candidacidal activity of phagocytic cells from normal and C5-deficient mice is equivalent (229), indicating that the opsonizing and/or chemotactic properties of C5 are important factors in the process of containment and elimination of the yeast. An intact complement system contributes to the early inhibition of the growth of *C. albicans* in normal and immune congenic resistant B10.D2 mice (200), but the presence or absence of C5 does not affect the development of specific immune responses or the ultimate outcome of challenge. Similar results were obtained in studies of experimental cutaneous candidiasis in C5-deficient mice (357), in that complement-deficient animals took longer to clear the infections, but the epidermal neutrophilic infiltrate in the skin of these animals was equivalent to that in the normal animals. When the physical barrier presented by the stratum corneum is penetrated, complement mediates an acute neutrophilic pustular response that restricts *Candida* proliferation and prevents further invasion of the tissue (266).

C5-deficient inbred mice show higher levels of tissue colonization than C5-sufficient strains (16), and a comparison of brain abscesses in DBA/1 ($C5^1$) and DBA/2 ($C5^0$) mice showed a relative paucity of inflammatory cells at the site of the lesions in the latter strain. These effects apparently depend on an absolute rather than a quantitative complement deficiency, as female mice, which have significantly lower concentrations of serum C5 than males, are markedly more resistant to infection (19, 108, 271). Although a deficiency in C5 would be expected to have similar consequences in different tissues and organs, the effect is most dramatic in the kidney (Table 4). A/J and DBA/2 mice, both C5 deficient, showed the highest colony counts in the kidney after sublethal challenge (17), but the kinetics of growth and clearance were similar to those in other strains. The fungal burden in the kidney of AKR mice, which also lack C5, was less than that in A/J and DBA/2 mice but greater than in C5-sufficient strains. These data suggest that mortality in A/J and DBA/2 mice is related to an unusual susceptibility of the kidney to colonization by *C. albicans* as-

sociated with tissue-specific differences in host protective mechanisms.

Summary. The discrepancies between measures of infection within and between strains can be interpreted as reflecting a hierarchy in the innate susceptibility to colonization and in the efficacy of the protective host response of different tissues and organs. At very low challenge doses, differences in the fungal load are seen only in the most susceptible organs, but as the inoculum is increased, discrepancies between organs and strains become apparent. Furthermore, if the excessive mortality of strains such as DBA/2 and A/J is caused by an accumulation of *Candida* organisms in the kidney, leading to renal failure, then survival may be increased by augmentation of local phagocytic defense mechanisms. However, this gross effect may not necessarily be linked to or reflect protective events occurring in other tissues.

HOST RESPONSE TO INFECTION

Role of Phagocytic Cells

PMNL. In the presence of fresh normal serum, mouse peripheral blood leukocytes and peritoneal macrophages ingest viable *C. albicans* blastoconidia at the same rate, but leukocytes kill intracellular yeasts more effectively than macrophages (172). In concordance with the situation in human candidiasis, an analysis of granulocytopenic and monocytopenic mice has confirmed that granulocytes rather than monocytes or exudate macrophages play a dominant role in the early host response against systemic *C. albicans* infection (337). There was little difference in functional activity between mouse PMNL isolated from the blood and those elicited by inflammatory stimuli such as caseinate and proteose-peptone (58); however, the latter display significantly greater candidacidal activity than those elicited by thioglycolate. In both murine and human PMNL, the most efficient system for the killing of *C. albicans* yeasts appears to be the myeloperoxidase-hydrogen peroxide-halide pathway. The amount of hydrogen peroxide released by mouse PMNL cultured with opsonized or unopsonized killed blastospores was proportional to the number of yeasts to which the cells were exposed (153), but the converse was found after incubation with viable organisms, even though the PMNL produced a stronger metabolic burst after stimulation. Thus, it appears that live blastospores are able to alter or inhibit the release of hydrogen peroxide by the phagocytic cells. Initiation of the oxidative metabolic burst and hydrogen peroxide production by the PMNL was found to be a property of the *C. albicans* mannan (92) and occurred via a mannose-inhibitable reaction pathway.

On the other hand, PMNL activated by *Blastomyces dermatitidis* antigen in vivo respond not only to this fungus but also to *C. albicans* by an increased oxidative burst and significantly enhanced killing (61), suggesting that optimal phagocytosis is attained in association with antigen-driven immunological reactivity. This is now widely recognized as being due to activation of the cells in vivo by cytokines produced by macrophages or other leukocytes. As in humans, both mouse PMNL (231) and macrophages (59, 60) can be activated for *Candida* killing in vitro by IFN-γ and other T-cell-derived cytokines.

Macrophages. Macrophages, too, make an important contribution to the clearance of *Candida* infections, and histopathological studies clearly indicate that an abundance of tissue phagocytes can preserve various organs from overt infection. For example, after intravenous injection in mice, the majority of the inoculum lodges in the liver and lungs (21, 251), yet these organs rarely sustain even minor damage, probably because of their large populations of resident phagocytes. In pulmonary candidiasis, a rapid accumulation of neutrophils in the lungs is essential for complete clearance of the yeast (193, 242), but studies with immunosuppressed mice showed that alveolar macrophages are required for both clearance and inhibition of dissemination of *C. albicans* from the lungs (301). Resident alveolar macrophages were highly efficient in killing *Candida* blastospores in vitro (300), although a comparison of inbred strains showed that this effector activity was a property of macrophages from mice bred in the C57BL/6 background.

There is, however, considerable heterogeneity in the effector function of macrophages derived from different anatomical sites. The candidastatic activity of macrophages freshly isolated from spleen was equivalent to that of Kuppfer cells and alveolar macrophages (96), while that of peritoneal macrophages was minimal. When cell populations from these same organs were matured in vitro in the presence of M-CSF, only macrophages from liver and spleen displayed significant candidastatic activity. Neither fresh nor differentiated macrophage preparations were as effective in inhibiting *Candida* growth as were cells derived from bone marrow after 3 days in liquid culture in the presence of M-CSF. The effector cells killed *Candida* blastospores by an extracellular mechanism and also displayed cytotoxic activity against NK-sensitive but not NK-resistant cell lines. These and previous studies (34, 95) suggest that the effector cell is a monocyte at an early stage of differentiation, and this may account for the failure to observe enhanced candidastatic activity after treatment of the effector cells with IFN-γ (96).

Nonspecific activation of macrophages, for example, by *Mycobacterium bovis* BCG purified protein derivative (BCG/PPD) in vitro, increases killing of phagocytosed *C. albicans* and inhibits the intracellular formation of germ tubes (338). In vivo, the number of *Candida* organisms in the kidney, spleen, and liver after intravenous injection is significantly lower in BCG/PPD-treated mice than in control mice. Augmentation of *Candida* killing was also obtained by preincubation of exudate but not resident macrophages with M-CSF (176). Although M-CSF induces exudate macrophages to produce IFN-α/β, the augmentation of candidacidal activity could not be blocked by an antiserum against IFN-α/β. In contrast, the candidacidal activity of resident peritoneal macrophages was enhanced by pretreatment with IFN-γ for 24 h (351), and the enhanced activity correlated with increased acidification of the phagolysosomes, suggesting that it was mediated by a proteinaceous substance(s).

Mouse peritoneal and splenic macrophages treated with IFN-γ in vitro expressed high candidacidal activity (75) that correlated with increased concentrations of nitrite in the culture supernatants. In these experiments, fungal killing and nitric oxide secretion by the activated macrophages were inhibited in a dose-dependent manner by IL-4 and IL-10, either separately or in combination. Peritoneal cells from mice infected intravenously with *C. albicans* showed an increase in nitric oxide synthesis that corresponded with the candidacidal activity of the cells (268), and nitric oxide has also been implicated in the resistance of mice with the severe combined immunodeficiency syndrome (*scid* mutant mice) to mucosal candidiasis (341). There is not, however, a direct correlation between nitric oxide production and *Candida* killing (340). These authors suggested that nitric oxide was candidastatic rather than candidacidal and was associated with or induced other macrophage candidacidal mechanisms. This apparent discrepancy may be associated with the different susceptibilities of yeast and hyphal growth forms to nitric oxide-mediated macrophage cytotoxicity (53).

The role in pathogenicity of the dimorphic or, more cor-

rectly, pleiomorphic (244) growth forms of *C. albicans* is still imperfectly understood, but there is increasing evidence that the yeast and hyphal forms not only differ in their susceptibility to killing and macrophage proteolytic activity (52) but also elicit functionally different responses from macrophages and macrophage cell lines. A cloned bone marrow-derived macrophage cell line responded to the hyphal form but not to the yeast form by an increase in TNF-α production that was not dependent on ingestion of the fungus by the macrophage (54). Neutralization of the TNF by the use of a polyclonal antibody resulted in a time-dependent decrease in TNF transcripts (51), showing that TNF was able to regulate its own production. It also influenced the synthesis of other cytokines by the macrophages. A macrophage cell line derived from the peritoneum also produced TNF after stimulation with hyphae but not yeasts, whereas a line from the lung responded to both (55). Although cells of microglial origin killed *Candida* organisms as efficiently as cells from other anatomical regions, they did not produce TNF after activation with either growth form. This suggests that secretion of TNF-α, because of its deleterious effects, may be under much tighter regulation in the brain than in other tissues.

Macrophages freshly isolated from tissues of mice showed patterns of responsiveness similar to those of the macrophage cell lines. Both resident and thioglycolate-elicited peritoneal macrophages produced TNF in response to stimulation with the hyphal but not the yeast form of the fungus (56), whereas splenic macrophages and PMNL responded to both, and bone marrow-derived macrophages responded to neither. Alveolar macrophages were also triggered to produce TNF-α by a *C. albicans* mannan (137). These observations indicate that the path taken by the developing immune response may be determined to some extent by the morphogenetic status of the fungus at the time that it interacts with the macrophage or antigen-presenting cell and the anatomical site where these early interactions occur.

Role of NK and LAK Cells

Although NK cells are able to kill *Cryptococcus neoformans* (152) and some other fungi (194), there is little evidence that they have any direct or indirect role in recovery from *Candida* infection in mice. Beige mutant mice, which are deficient in NK cells, develop more severe lesions in the brain and other tissues than their heterozygous littermates, but the severity of the lesions is independent of the magnitude of the NK defect (26) and is probably related to some functional defect in the PMNL in the homozygous mice (35). Furthermore, *scid* mutant mice, which are deficient in both T and B cells, display no enhanced susceptibility to infection after depletion of NK cells by treatment with anti-asialo-GM_1 (143), and conventional mice infected with an attenuated strain of *C. albicans* and depleted of NK cells by treatment with a monoclonal antibody showed no changes in the development of resistance to reinfection or in Th cell function (285).

Nevertheless, *C. albicans* does stimulate NK cell activity. A single intraperitoneal injection of inactivated *Candida* yeasts induces a subset of cells sensitive to the asialo-GM_1-specific antibody, which displays cytotoxic activity against YAC-1 cells (202). Multiple injections, on the other hand, generate a population of nonadherent, nonphagocytic, large granular lymphocytes that kill both NK-sensitive and NK-resistant tumor target cells in vitro (303) and display phenotypic and functional properties similar to those of LAK cells generated in vitro. Analysis of cytokine mRNA production by the peritoneal exudate cells at the conclusion of the course of injections (294) revealed IL-1β, IL-2, IL-6, IL-10, IFN-γ, and TNF-α but not IL-12. IL-2 was expressed and sustained at high levels; however, there was only weak, transient expression of mRNA for the other cytokines. Administration of a single dose of recombinant human IL-2 (rhIL-2) to the *Candida*-treated mice increased NK cell activity but did not activate LAK-like effectors (305), whereas rhIL-2 given in combination with the inactivated yeast strongly increased both the cellularity of the peritoneal exudate and the cytotoxic activity of the peritoneal NK and LAK cells (304). These LAK-like cells, induced either in vivo or in vitro, exhibit high levels of activity against *C. albicans* hyphae (302). The biological relevance of this phenomenon is unknown, although it has been suggested that it may represent a second level of host defense against *C. albicans* hyphal forms in immunosuppressed mice (302).

Effect of Cytokines In Vivo

As recombinant cytokines have become more readily available, they have increasingly been used to modify the host response to *C. albicans* infection in vivo. However, the effects of treatment have not always been consistent and are sometimes difficult to interpret.

Administration of recombinant IFN-γ (rIFN-γ) to infected mice was associated with significantly improved survival after lethal challenge (267), and this correlated with an increase in the candidacidal activity of peritoneal macrophages and Kupffer cells after treatment with IFN-γ in vitro. Another study of the effect of rIFN-γ in vivo showed a reduction in the growth of *C. albicans* in the kidneys, spleen, and liver of mice after treatment from 1 day before to 3 days after infection (190). In this case, the enhanced resistance of the treated mice correlated with a significant increase in the capacity of peripheral blood and peritoneal exudate granulocytes to kill *C. albicans* in vitro. In a different experimental model, treatment of mice with IFN-γ actually decreased resistance to challenge (136), although boosting immune responsiveness by immunization overrode the suppressive effects. Similarly, poly(I · C), a potent inducer of interferons in vivo, increased the susceptibility of CB-17 *scid* mice to acute systemic candidiasis and to systemic candidiasis of endogenous origin (164). The enhanced susceptibility was abrogated by in vivo treatment with antibodies to IFN-α, -β, and -γ. The effect was independent of NK cells and was attributed to an impairment of the candidacidal activity of macrophages.

Cytokines can also enhance resistance to infection by stimulating the production of phagocytic effector cells. Administration of recombinant human IL-1 (rhIL-1) to mice did not significantly elevate blood neutrophil concentrations but substantially increased the number of PMNL within the tissues without causing detectable changes in macrophage numbers (191). The bactericidal capacity of splenocytes rose in parallel with PMNL accumulation. Comparable doses of TNF also enhanced killing of *Listeria monocytogenes* in vivo, but in contrast to IL-1, TNF significantly depressed peripheral blood neutrophil counts and inhibited the accumulation of neutrophils in the spleen. Other experiments confirmed that prophylactic treatment with rhIL-1β enhanced the resistance of mice to systemic *C. albicans* infection (253).

In mice myelosuppressed by cyclophosphamide, subcutaneously administered rGM-CSF increased the number of precursor cells in bone marrow and caused a profound neutrophilia that protected the mice against lethal infection with the yeast (217). Prophylactic treatment with human G-CSF (hG-CSF) conferred significant protection against systemic *C. albicans* infections in cyclophosphamide-treated (211) but not in corti-

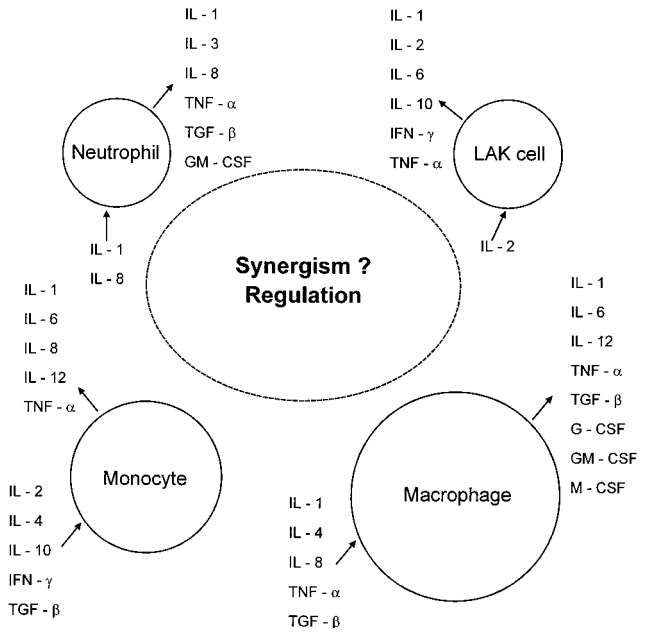

FIG. 1. Cytokine responses of immune and inflammatory cells. Only those cytokines presently known to be relevant to *C. albicans* infection are shown. The arrows pointing inward indicate cytokines that have an effect on the cell; those pointing outward indicate cytokines produced by the cell.

sone-treated mice (260). G-CSF increased production of neutrophils by bone marrow in vivo (132), thus augmenting resistance to challenge with *C. albicans* and other fungal pathogens (132, 335). It also acted synergistically with stem cell factor to stimulate granulocyte recovery and protect against lethal infection with *C. albicans* in cyclophosphamide-treated mice (321). However, localized candidiasis in neutropenic mice did not respond to hG-CSF (260).

There are conflicting reports on the role of M-CSF in murine candidiasis. After infection in [BALB/cCr × DBA/2Cr]F_1 hybrid mice, the number of monocytic precursor cells in the bone marrow increased, and both serum and tissue concentrations of M-CSF were elevated (73). Administration of the purified cytokine to infected mice reduced the number of colonies recovered from the organs and extended survival time. The beneficial effects in vivo correlated with an enhanced candidacidal activity of macrophages treated with M-CSF in vitro. On the other hand, treatment of C3H mice with M-CSF, either before or after intravenous infection with *C. albicans*, exacerbated the disease, causing a doubling of the rate of weight loss and leading to significantly earlier death (159). This result was interpreted as evidence favoring a major role for macrophages in the pathology of the disease, but recent results (see below) have raised the possibility that alterations in the cytokine milieu may have favored the development of different Th subsets in the different mouse strains.

It is, of course, not possible to resolve these conflicts without conducting experiments that control for variables such as mouse strain and the "virulence" of the yeast, but the discrepancies emphasize that the effects of administration of any individual cytokine in vivo will markedly influence and be influenced by the host microenvironment (Fig. 1). Furthermore, the introduced cytokine may enhance or inhibit the production of others, distorting the normal response to the infection and making unequivocal interpretation of the results very difficult. These issues will be considered at greater length within the context of cytokine regulation of the T-lymphocyte response to the infection.

IMMUNE RESPONSES TO *C. ALBICANS*

In general, experimental results in mouse models have suggested that cell-mediated and humoral types of immunity play major and minor roles, respectively, in host defense against *C. albicans* (173). However, in evaluating the relative contributions of the various effector mechanisms, it is crucial to make a clear distinction between those that are responsible for recovery from an initial (primary) infection and those that mediate protection from reinfection. Specifically, the presence of cell-mediated immune responses in an immune animal does not necessarily indicate that these are the cause of the enhanced resistance to rechallenge (28, 29).

Cell-Mediated Immune Responses

Establishing a role for T cells and cell-mediated immunity in *Candida* infections has been difficult, and early results were controversial. Although neonatally thymectomized mice showed an increased susceptibility to *C. albicans* infection (297), an effect of T-cell deficiency was not observed in adult thymectomized, irradiated, bone marrow-reconstituted AT X BM mice (140) or in nude mice (90, 277). The resistance of AT X BM and nude mice can probably be attributed to nonspecific activation of the monocyte/macrophage system (79, 364) and appears to be short-lived, as both strains have been shown to be substantially more susceptible than controls in the later stages of the infection (227, 311).

Mice treated with a Thy-1.2-specific monoclonal antibody in vivo show a significant reduction in the efficiency of clearance of *Candida* organisms from the spleen (9), confirming that T lymphocytes contribute to recovery from primary infection, and these findings are consistent with the further demonstration that host responsiveness in congenic resistant mice is regulated by genes within the MHC (9, 10). Corroborative evidence was provided by the transfer of a *Candida*-specific T-cell line into sublethally irradiated mice (309); the T-cell line conferred resistance against primary systemic challenge. There is evidence that both $CD4^+$ and $CD8^+$ cells contribute to the host response against infection (13, 76, 83), and the general thrust of results to date indicates that a central element of host resistance is the production of cytokines by *Candida*-specific T cells.

The specificity of both $CD4^+$ and $CD8^+$ T lymphocytes is defined during differentiation in the thymus, and this organ has also been thought to be the source of a family of hormone-like products (thymosins) important to the maintenance and functioning of the immune system. Therefore, early attempts at modulating and enhancing host resistance to *C. albicans* involved treatment of mice with these thymic "hormones."

Effect of Thymosin

Thymosin fraction V is a partially purified extract of calf thymus containing a complex mixture of 40 to 60 peptides with molecular masses of less than 10 kDa. Several of its components possess immunopotentiating activity and mediate a variety of effects on cell-mediated immune responses.

In the present context, thymosin fraction V has been used to modulate host responses to *C. albicans* in inbred strains of mice that are either resistant or susceptible to intravenous challenge. The different strains showed a direct correlation between colony counts in the kidney and levels of macrophage inhibitory factor and IFN-γ released into the circulation (238).

Administration of thymosin fraction V beginning on the day of infection greatly enhanced the resistance of the susceptible strains but increased levels of infection in the resistant strains (296). In parallel with this, cytokine production by the susceptible and resistant strains was increased and reduced, respectively (238). In contrast, treatment with thymosin factor V increased specific DTH responses of resistant mice but had no effect on delayed footpad reactions in the susceptible strains (296). The impairment of cell-mediated immune responses in mice made diabetic with alloxan could also be reversed by thymosin factor V (298).

Thymosin α_1 was the first of the thymic peptides to be purified to homogeneity (327) and showed many of the biological properties of thymosin fraction V. Treatment of mice with thymosin α_1 conferred protection against lethal intravenous infection with *C. albicans* if administered before but not after challenge (45) and also prevented the increased susceptibility to infection caused by cyclophosphamide. The protective effect was associated with an increase in both the numbers and the candidacidal activity of circulating PMNL in the peripheral blood (43). This therapeutic approach was found to be effective when used either alone or in combination with fluconazole in ameliorating systemic candidiasis in mice immunosuppressed by treatment with morphine (99). The protective effect of thymosins in vivo is probably mediated by their ability to stimulate the production of a variety of different cytokines (30, 158, 186).

The mechanisms of action of the various thymosins have not yet been elucidated. Thymosin α_1 has been shown not to be a hormone (327) but is instead a breakdown product of an abundant cellular protein called prothymosin, which exhibits many of the same biological and immunological properties but whose primary function appears to be the regulation of cellular proliferation. Thymosin β_4 is another well-characterized constituent of thymosin fraction V that also exhibits immunoregulatory activities, but these too may be secondary to its actin-sequestering properties and potential role in the regulation of the microfilament system (225). The whole issue is rendered much more complex by evidence that the thymosins function as immunotransmitters (146), conveying signals both directly to leukocytes and indirectly to the immune and other homeostatic systems via neuroendocrine and autonomic nerve pathways. Thus, they may function not only to stimulate specific effector mechanisms but also to coordinate the whole-body response to infection or disease.

Candidiasis in Mutant Mice

Further evidence consistent with an important role for T lymphocytes in murine candidiasis comes from studies of mucosal infections in germfree athymic mice. After colonization, lymphocyte proliferation and footpad responses to *Candida* antigens were demonstrable in the *nu*/+ control mice, and these manifestations of cell-mediated immunity correlated with the clearance of *Candida* hyphae from the dorsal surface of the tongue and from the stomach (37). Conversely, *nu*/*nu* mice could not clear mucosal candidiasis and did not exhibit *Candida*-specific lymphocyte proliferation or footpad swelling. The *nu*/*nu* mice did not develop a progressive systemic disease, indicating that "nonimmune" mechanisms were sufficient to prevent dissemination from the gastrointestinal tract in this model.

Investigations of the effect of the combined beige (*bg*/*bg*) and nude (*nu*/*nu*) mutations on the development of systemic candidiasis after gastrointestinal colonization (64, 65) revealed that only the beige athymic (*bg*/*bg nu*/*nu*) mice developed severe infections. In contrast, *bg*/*bg* mice, *nu*/*nu* mice, and *bg*/*bg nu*/*nu* mice were all resistant to naturally occurring vulvovaginal candidiasis (63). *Candida*-colonized beige euthymic but not beige athymic mice developed *C. albicans*-specific antibody and cell-mediated immune responses. The susceptibility to candidiasis of *bg*/*bg* mice is probably attributable not to a deficiency of NK cells (26) but to some reduction either in the effector function of the PMNL (168, 189) or in the ability of macrophages to respond to activation by cytokines (339). These data suggest that a combination of defects in both phagocytic cell function and cell-mediated immune responses predispose mice to severe mucosal and systemic candidiasis of endogenous origin.

Mutant *scid* mice lack functional T and B cells, but neither conventional (201) nor germfree (38) animals display any unusual susceptibility to intravenous challenge with *C. albicans*. However, gastrointestinal candidiasis was more easily established in *scid* mice than in controls (234), and when they were treated with a monoclonal antibody to murine granulocytes (anti-Gr-1), with silica, or with carrageenan, *scid* mice showed enhanced susceptibility to acute systemic candidiasis, disseminated candidiasis of endogenous origin, and orogastric candidiasis (165). These data are consistent with clinical studies that point out the important role played by granulocytes in host defenses against *Candida* infections; nevertheless, macrophages (166) as well as T and B cells (165) have also been shown to contribute to the control of orogastric and disseminated candidiasis of endogenous origin.

Genetic Regulation of Host Immune Responses

Inbred strains of mice that develop severe (type I) or mild (type II) lesions in the tissues [21] can be independently categorized as producing high or low immune/inflammatory responses in the popliteal lymph nodes, respectively, after challenge with viable *C. albicans* blastospores. Thus, CBA/H mice are type I and low responders, whereas BALB/c mice are type II and high responders (9). During primary infection with *C. albicans*, the magnitude of T-lymphocyte responses in the draining lymph nodes is determined by genes within the mouse MHC (9), and these host immune/inflammatory responses are controlled, at least in part, by class I MHC genes (10). However, in comparison to the BALB/c strain, congenic resistant BALB/k ($H\text{-}2^k$) mice show a significantly reduced inflammatory response in the popliteal lymph node after footpad immunization (9), even though they exhibit mild (type II) lesions.

Analysis of lymph node responses in (BALB/c $\times$ BALB/k)F_1 hybrid mice shows that low responsiveness is dominant (23); however, a background gene(s) present in B10 congenic resistant strains appears to either modify or override the MHC-linked regulation of host responses (9). The regulatory effect of MHC genes is substantially weaker than that of background genes, which generally determine the outcome of infection in infectious disease models; but MHC genes have been linked to diseases in which there is a significant immunopathological component (365). Nevertheless, in *Candida* infection, the magnitude of primary T-lymphocyte responses does not correlate with the severity of lesions in different inbred strains of mice, suggesting that the MHC associations influence other aspects of the host immune response.

Cytokine Production by T-Cell Subsets

The major function of the T lymphocytes in *Candida* infection is the production of cytokines that enhance the candidacidal activity of mononuclear and polymorphonuclear effector cells. T cells can be divided into two functionally distinct

subpopulations on the basis of the presence or absence of CD4 and CD8 antigens or the cell surface, and in the mouse at least, $CD4^+$ Th cells can be separated into distinct groups according to their patterns of cytokine production.

Originally, two main subgroups were identified—Th_1 cells, secreting IL-2 and IFN-γ, and Th_2 cells, secreting IL-4 and IL-5 (233). The mode of antigen presentation influenced the development of the Th subsets, in that antigen presentation by splenic B cells favored the Th_2 subpopulation, while presentation by macrophages favored the generation of Th_1 cells (133). In a mouse model of leishmaniasis, clones belonging to the different Th subsets were found to respond to different parasite antigens and to subserve different roles (307), and there was reciprocal expression of mRNAs for IFN-γ and IL-4 in the spleen and lymph nodes of *Leishmania*-infected C57BL/6 and BALB/c mice, which are resistant and susceptible, respectively, to infection (150). However, a survey of inbred strains (230) has shown that, along the spectrum of cutaneous leishmaniasis, IL-4 is a reliable indicator of disease but IFN-γ does not correlate with resistance.

There has been a degree of controversy about the relevance in vivo of a classification based on T-cell clones, in that patterns of cytokine production by individual alloreactive T cells are essentially random (179) and there exist clones that coexpress cytokines "diagnostic" of both Th subsets. However, the demonstration that human $CD4^+$ lymphocytes also develop stable Th_1 and Th_2 cytokine profiles (281) suggests that the phenomenon is general, and recent data (255) have shown that there are differences in cytokine receptor signaling pathways between committed cells of the Th_1 and Th_2 subsets. The implication from these studies at the clonal level is that infectious agents, such as *Leishmania* spp., induce an immune response that exhibits, at the population level, a dominance of T cells expressing a Th_1- or Th_2-like pattern of cytokine production.

This paradigm has had important ramifications in the analysis of *Candida* infections, particularly as cytokines typical of the two subsets not only augment or depress the function of phagocytic cells but also regulate crucial pathways in the immune response.

Cellular Immune Responses in Mucosal Infection

Candida infection of the oral mucosa in mice triggers an inflammatory response and stimulates cellular immunity (192). Following a second topical challenge with *C. albicans*, the infection was barely detectable, and a typical DTH reaction occurred at the site. Similar effects were seen after gastrointestinal infection of infant mice (108), in that colonization primed the mice for enhanced DTH responses and protected adult mice from systemic challenge.

The influence of the genetic background of the mice on the development and resolution of mucosal infection was examined in BALB/c and DBA/2 mice, which carry the same MHC haplotype ($H\text{-}2^d$). After topical administration of *C. albicans* to the oral mucosa, both strains showed an increase in $MAC\text{-}1^+$ cells and a similar recruitment of $CD4^+$ and $CD8^+$ T lymphocytes into the mucosal tissue (78). Infection was associated with an influx of γ/δ T cells on day 3 in BALB/c mice but on day 5 in the DBA/2 strain, which coincided with a sudden decrease in the number of viable organisms recovered from the mucosal tissue. The two strains also differed in the sequelae of infection, in that systemic immunity, demonstrable as *Candida*-specific DTH responses, developed in the DBA/2 but not in the BALB/c mice. There is a curious parallel here with the expression of strong antibody-mediated protective responses in CBA/CaH mice, which develop severe tissue damage, compared with a less obvious effect in the more resistant BALB/c strain (22).

A functional role for the γ/δ T-cell subset was also demonstrated by intraperitoneal infection of immunocompetent mice and knockout mice (mice with targeted gene deletions) with *C. albicans*. This resulted in a steady accumulation of both γ/δ T cells and macrophages in the peritoneal cavity (169). The γ/δ T cells produced IFN-γ, which enhanced macrophage candidacidal activity and nitric oxide production. This T-cell subset may represent an important element in host resistance, as specific depletion of these cells increased the susceptibility to mucosal candidiasis of both immunocompetent and T-cell-deficient (*nu/nu*) mice. Nevertheless, α/β T cells have also been implicated in host defense. Previous experiments by the same group had demonstrated that mucosal *C. albicans* infection in the alimentary tract of multiply immunodeficient (*bg/bg nu/+*) mice induced a population of lymphocytes in both the Peyer's patches and spleen that proliferated and produced IL-2 in response to stimulation with *Candida* antigens (66). *Candida*-reactive lymphocytes were not found in mice homozygous for the nude mutation (*bg/bg nu/nu*), and these animals were unable to clear the yeasts. The presence of these lymphocytes correlated with clearance of the yeast from the esophagus and tongue. Depletion of $CD4^+$ cells increased susceptibility, but neither IL-2 nor IFN-γ could be identified as a mediator in this experimental system.

Other models of mucosal infection have placed a greater emphasis on the function of cytokines associated with the different Th subsets. Although DBA/2Cr mice develop fatal disseminated candidiasis after intravenous infection with the avirulent PCA-2 vaccine strain (284), intragastric colonization of these mice with the virulent CA-2 strain was associated with the induction of strong cell-mediated immune responses of the Th_1 type and eventual clearance of the infection (44). In BALB/c mice, which are resistant to systemic challenge with the PCA-2 yeast strain (284), gastrointestinal colonization with the CA-2 yeast strain resulted in the production of both Th_1-type (IFN-γ) and Th_2-type (IL-4 and IL-5) cytokines by $CD4^+$ cells from Peyer's patches and mesenteric lymph nodes at a time when the yeasts were being cleared from the intestine (74). Administration of recombinant IL-4 or IL-10 to the mice exacerbated the course of infection (331) and induced production of high levels of these same cytokines by $CD4^+$ lymphocytes from the Peyer's patches. In contrast, enhancement of mucosal Th_2 responses by oral administration of cholera toxin did not modify the course of the disease, but intravenous treatment of the infected mice with soluble IL-4 receptor, which enhances Th_1-type cytokine responses (261), increased gastrointestinal clearance of the yeasts.

Vaginal *Candida* infection in CBA/J mice induced systemic cell-mediated immunity within 2 weeks (126), but in vitro analysis of cytokine production by lymph node cells detected predominantly IL-2 and IFN-γ, with no IL-10, and only small elevations of IL-4 in the later stages of infection. Rather unexpectedly, the course of primary vaginal candidiasis was unaffected by preinduction of either systemic immunity or *Candida*-specific suppressor cells (127), even though the latter significantly reduced the DTH responses elicited by the vaginal challenge. However, when mice in which Th_1-type systemic responses elicited by an initial vaginal infection were given a second vaginal inoculation, they developed anamnestic DTH responses and showed reduced colonization of the vagina compared with mice with primary infections (123). Although severe oral candidiasis can occur in association with an immunodeficiency characterized by a reduction in $CD4^+$ T cells in the

absence of human immunodeficiency virus infection (138, 250), monoclonal antibody depletion of $CD4^+$ and $CD8^+$ cells had no effect on primary or secondary vaginal candidiasis in mice (128).

The general conclusion from these results is that resistance to mucosal infections is determined by phagocytic mechanisms, the activity of which is augmented or reinforced by Th_1-type cytokines. Although there is some evidence of a link between the mucosal and systemic immune responses, specific pathways have not yet been identified. Cross-regulation of *Candida*-specific immune responses has been examined in greater detail using models of systemic infection.

Cytokines in Vaccine-Induced Resistance

Infection of mice with an attenuated strain of *C. albicans* (PCA-2) incapable of yeast-mycelium transitions was found to increase resistance against a subsequent challenge with the virulent parental strain (CA-2) (46). The protection induced was nonspecific and depended on the inoculation of sufficient organisms to establish a long-lasting chronic infection without causing death (344). Anti-infectious activity was directly correlated with macrophage activation (46, 344) and appeared to be independent of the function of T lymphocytes, as the effects could be reproduced in athymic mice (47). After treatment with PCA-2, high levels of GM-CSF, TNF-α, IL-1, and IFN-γ were detected in the serum and in the supernatants of spleen cell cultures (342), and cytokine production was also correlated with the expression of microbicidal activity in vivo.

In normal (euthymic) mice, infection with the PCA-2 strain elicited strong DTH reactions to *C. albicans* concomitantly with the expression of candidacidal activity by activated splenic macrophages (76). The DTH reactivity was attributable to $CD4^+$ T lymphocytes that produced IFN-γ and GM-CSF in vitro. Elimination of the $CD4^+$ cells by treatment of the mice with a monoclonal antiserum abrogated the DTH response and increased the burden of PCA-2 yeasts in the kidneys (76) but did not alter the survival of mice challenged with strain CA-2. This result is consistent with previous work (9) showing that depletion of T lymphocytes from sublethally infected mice impairs clearance of the yeast partially but not completely and confirms the importance of activated macrophages in augmenting resistance against lethal challenge in this experimental model (46, 47).

The contribution of the $CD4^+$ and $CD8^+$ T-cell subsets to the development of immune responses and protection against lethal challenge after priming with the attenuated PCA-2 strain was evaluated by administration of antibodies to CD4, CD8, or IFN-γ in the early stages of infection (283). Treatment with antiserum to either CD4 or IFN-γ modified the course of the disease and prevented the development of resistance to reinfection, but mice given a combination of both antisera developed a fatal candidiasis caused by overgrowth of the attenuated vaccine strain. In vitro, spleen cells from PCA-2-primed mice produced high levels of IFN-γ in response to stimulation with *Candida* antigen (77), and this activity was abolished only by complement-mediated lysis of the responder population with antisera to both $CD4^+$ and $CD8^+$ lymphocytes. Purification of $CD4^+$ and $CD8^+$ cells by positive selection confirmed that both produced IFN-γ provided that appropriate accessory cells were present during in vitro stimulation with the yeast. Resistance to challenge with the virulent CA-2 yeast strain induced by prior infection with the PCA-2 strain was impaired either by depletion of $CD4^+$ and $CD8^+$ cells or by treatment of the primed mice with a polyclonal antiserum to IFN-γ (77).

These two studies demonstrated that $CD4^+$ T cells play the dominant role in the early evolution of protective responses induced by infection with the avirulent yeast strain but that both $CD4^+$ and $CD8^+$ cells are involved in the expression of acquired resistance to subsequent challenge. IFN-γ is involved in both the early and late phases of the response. Further analysis of this model has focused on the role of the $CD4^+$ Th-cell subsets in the evolution of host responses.

Th Subsets in *Candida* Infection

When two agerminative variants of *C. albicans* produced by chemical mutagenesis were used to elicit anti-infectious protection, different responses were obtained in vivo and in vitro. One variant induced significant resistance against challenge with the virulent parental strain that was associated with DTH responses in vivo and increased the fungicidal activity of macrophages in vitro (289). The other variant was not protective. The protective variant induced $CD4^+$ lymphocytes that released large amounts of the Th_1 cytokines IFN-γ and IL-2, whereas $CD4^+$ cells generated by immunization with the nonprotective variant produced the Th_2 cytokines IL-4 and IL-6. The connection between Th type and disease outcome was elegantly demonstrated by Romani et al. (282), who showed that a single injection of a monoclonal antibody to IFN-γ in conjunction with the PCA-2 yeast strain caused a change in the cytokine profile from a Th_1-type, associated with protection, to a nonprotective Th_2-type response.

Similar reciprocity between Th_1 and Th_2 cytokine profiles and induction of resistance or progression of the disease was observed in BALB/cCr and DBA/2Cr mice after infection with the PCA-2 vaccine strain of *C. albicans* (284). As is the case in the (BALB/cCr × DBA/2Cr)F_1 hybrid (76, 342), BALB/cCr mice developed resistance to reinfection associated with Th_1 responses, whereas DBA/2Cr mice exhibited a progressive disease and showed sustained production of Th_2 cytokines by $CD4^+$ cells in vitro (284). However, when the DBA/2 mice were treated with an IL-10-specific antiserum at the time of infection, they survived (292). Recovery was associated with the onset of footpad responses and the development of durable protection from reinfection. In vitro, the level of expression of IL-4 and IL-10 mRNAs in $CD4^+$ spleen lymphocytes was reduced, and macrophages displayed increased production of nitric oxide after activation by IFN-γ. Similarly, when (BALB/cCr × DBA/2Cr)F_1 hybrid mice were infected with the virulent CA-2 strain of *C. albicans* and treated with a monoclonal antibody to IL-4, a high proportion of the mice survived (286). Disease resolution was evinced by clearance of the yeast from infected organs and was associated with strong Th_1 responses and the establishment of long-lasting protective immunity. Reciprocal changes in cytokine mRNA levels were seen in $CD4^+$ cells, in that there was a decrease in IL-4 mRNA and a corresponding increase in IFN-γ transcripts. Identical results were obtained by treatment of the infected mice with recombinant soluble IL-4 receptor (261).

Comparison of cytokine responses in mouse-yeast strain combinations that induce either self-limiting infections and survival (healer) or chronic disease and death (nonhealer) showed that IL-4 and IL-10 production correlates with progressive fatal infection. In both healer and nonhealer combinations, administration of IL-4 or IL-10 accentuated the severity of infection and led to a progressive dominance of the Th_2 pattern of lymphocyte reactivity (331). Secretion of IFN-γ protein by $CD4^+$ and $CD8^+$ lymphocytes occurred only in mice with self-limiting infections (288), but high levels of IFN-γ were detected in the serum of mice that succumbed to infection, thus excluding a defect in the IFN-γ response as a factor

that biased these mice towards a preferential expansion of Th_2 cells. In mice that recovered from infection, IL-12 mRNA was continuously expressed in splenic macrophages, and it was also required for *Candida*-specific induction of IFN-γ by $CD4^+$ cells in vitro. In subsequent experiments, its role in driving Th_1 differentiation in vivo was investigated by treating mice with either the neutralizing antibody or the recombinant cytokine (287). Mice with self-limiting infections did not develop resistance after neutralization of IL-12 and showed Th_2-type responses. However, when mice suffering from progressive disease were treated with rIL-12, they did not recover or exhibit any change in Th profile.

Although healer and nonhealer responses have been linked to the activities of cytokines that reflect the preferential expansion of Th_1 and Th_2 lymphocyte subpopulations, other cytokines play an important role in host resistance and in the evolution of protective immunity. TGF-β modulates the function of T and B lymphocytes and was found to be upregulated in mice with self-limiting infections but downregulated in animals that succumbed to systemic challenge (318). Neutralization of TGF-β in vivo did not affect the outcome of primary challenge in a healer combination, but it compromised the development of resistance by these mice against a later challenge with a virulent yeast strain. However, the role of this cytokine is not clearcut, as treatment of nonhealer mice with recombinant TGF-β delayed progression of the disease.

The studies described above have demonstrated that the balance between a Th_1- and a Th_2-type response is labile and capable of being influenced by the virulence of the yeast strain and the genetic context in which infection takes place. Perhaps not surprisingly, other, extraneous factors can also influence disease outcome and Th phenotype. For example, outbred mice made diabetic by treatment with streptozotocin developed an acute lethal infection after challenge with the low-virulence PCA-2 strain of *C. albicans* (232), and the disease was associated with the production in vitro of a Th_2-like cytokine profile by $CD4^+$ lymphocytes (221). Unfortunately, the mechanisms that determine the dominance of the Th_2 phenotype in this model have yet to be elucidated.

Synergistic Effects of Concomitant Infection

Coinfection of mice with *C. albicans* and murine cytomegalovirus (147) or some bacteria (68) causes a synergistic interaction that results in exacerbation of infection and increased mortality. In a combined murine cytomegalovirus-*C. albicans* infection (148), the titers of murine cytomegalovirus in tissues were unaltered, but the *C. albicans* infection changed from a self-limiting to a fatal progressive disease. Similarly, administration of *Escherichia coli* or *E. coli* lipopolysaccharide to mice given a lethal inoculum of *C. albicans* increased kidney colonization and accelerated death (1). In contrast, when various *Staphylococcus aureus* isolates were administered to mice in conjunction with a sublethal dose of *C. albicans*, the severity of the bacterial infection increased dramatically (70), especially in mice receiving *S. aureus* strains derived from patients with toxic shock syndrome (69).

S. aureus contains an enterotoxin that belongs to a family of powerful T-cell stimulants called superantigens, which interact directly with specific alleles of the Vβ chain of the T-cell receptor (356). Activation by superantigens can lead to clonal expansion, deletion, or anergy of the target T-cell subset (243). Treatment of mice with staphylococcal enterotoxin B leads to the induction of anergy in $V\beta8^+$ $CD4^+$ lymphocytes (177) and biases the cytokine response towards a Th_1 type (39). The gradual development of anergy in the $V\beta8^+$ subpopulation correlates with a decrease in IL-4 and reciprocal increase in IFN-γ production by $CD4^+$ lymphocytes (293), accompanied by an enhanced resistance against lethal systemic challenge with *C. albicans*. In contrast, when the mice were primed with staphylococcal enterotoxin B and challenged with *C. albicans* within 24 h, mean survival times were dramatically shortened (293). The relevance of this latter result to the natural course of infection is unclear, as coinfection with *S. aureus* does not lead to a synergistic enhancement of *C. albicans* infection (70) and T cells in the lesions of human patients with oral candidiasis do not express the biased T-cell receptor distribution that would be consistent with the involvement of a superantigen (310).

TABLE 5. Effect of cytokine and anticytokine treatment on resistance of mice to *C. albicans* infection[a]

Cytokine or anticytokine	Effect on resistance
IFN-γ	Increased
Anti-IFN-γ	Decreased
IL-12	Decreased
Anti-IL-12	Decreased
IL-4	Decreased
Anti-IL-4	Increased
IL-10	Decreased
Anti-IL-10	Increased

[a] Reproduced with permission from Mencacci et al. (220).

In summary, by using combinations of wild-type and avirulent yeast strains in susceptible and resistant strains of mice, this experimental system has illuminated the pathways by which characteristic cytokine profiles develop within the Th_1/Th_2 paradigm and the ways in which they can be modified by the use of appropriate reagents (Table 5). Nevertheless, it is clear that there are anomalies that involve not only the role of $CD4^+$ and $CD8^+$ T lymphocytes but also the contributions of other cytokines.

Immunopathology in Primary Systemic Infection

All of the studies reviewed thus far have found that $CD4^+$ lymphocytes are the primary T-cell subset involved in the generation of cell-mediated immune responses to *C. albicans*. A quite different outcome was obtained after treatment of acute systemic infection in BALB/c mice with monoclonal antibodies specific for $CD4^+$ or $CD8^+$ cells (83).

In these experiments, depletion of $CD8^+$ cells did not influence either overall survival or mean survival time, whereas depletion of $CD4^+$ cells significantly enhanced both. Combined depletion of $CD4^+$ and $CD8^+$ cells significantly lengthened the mean survival time, although it did not increase the proportion of animals that survived. These data demonstrated that $CD8^+$ cells could exert a beneficial effect in recovery from primary infection but that this was overshadowed by a stronger immunopathologic $CD4^+$ response. The protective effect of CD4 depletion was not associated with functional changes in phagocytic cells, so it was suggested that lethality might be due to the production of potentially harmful cytokines, such as TNF.

Intraperitoneal infection with *C. albicans* in mice raises levels of endogenous TNF (273) and causes a chronic elevation of plasma fibrinogen levels without altering other blood parameters. The possible contribution of TNF-α to early death in acute *C. albicans* infection was evaluated in BALB/c mice (3). After administration of 10^8 blastoconidia, mice died within 12 h. The TNF concentrations in sera from these mice were significantly greater than in controls, but pretreatment with a

TNF-α-specific antiserum did not reduce mortality. In contrast, mice given murine TNF-α were more resistant to challenge, indicating that, far from mediating a toxic shock-like process, TNF-α may enhance phagocytic clearance of the yeast. In a less aggressive experimental model (198), TNF-α was first detected in the serum 16 h after infection. Concentrations increased progressively and reached peak levels shortly before death. Administration of a polyclonal antibody against TNF-α increased colony counts in the tissues and decreased survival.

A protective effect of TNF-α was further demonstrated in normal and granulocytopenic mice (322). During *C. albicans* infection, both TNF-α and IL-6 were secreted into the serum in a dose-dependent manner. Growth of the yeasts in the kidney was significantly increased in normal mice treated with a monoclonal antibody against TNF-α, but the fungal burden in similarly treated granulocytopenic mice was unaffected, suggesting that TNF is essential for granulocyte antifungal activity in vivo. Somewhat different results were obtained after pulmonary infection in normal and cyclophosphamide-treated mice (132). The granulocytopenic mice developed high levels of TNF-α in serum and bronchoalveolar lavage fluid and died within 48 h, whereas the normal mice survived the challenge and had very low levels of TNF-α. Administration of G-CSF to the granulocytopenic mice markedly reduced TNF-α concentrations, increased the numbers of granulocytes, and significantly prolonged survival.

Clearly, considerable progress has been made in defining the cytokine-mediated effector pathways that contribute to protection from lethal systemic infection, but there are certainly points of conflict between results generated in different experimental systems, and many issues remain to be clarified. However, the most problematical area in human infection is not protection against lethal systemic infection, about which there is now a good understanding, but the issue of susceptibility to recurrent *Candida* vaginitis. As detailed above, numerous animal models of mucosal infection have been developed and characterized, but none have shed light on the defects that may predispose to the human disease. As recurrent candidiasis is often associated with unusually severe tissue inflammation (160), an alternative experimental approach has been used to analyze host responses in mice that show genetically determined differences in tissue responses to systemic infection.

The relevance of such a model to mucocutaneous disease can be questioned, but such qualms are probably groundless for the following reasons. First, there is considerable evidence that *C. albicans* can, under certain conditions, penetrate the mucosal (especially gastrointestinal) barrier in both normal humans (187) and unmodified mice (108, 116); second, gastrointestinal colonization induces systemic immunity (108, 125); and finally, chronic mucocutaneous candidiasis is strongly linked to defects in systemic cell-mediated immune responses (183).

Cytokine Production in Infected Tissue

In approaching an analysis of the variables that contribute to the expression of type I (severe) and type II (mild) lesions in different inbred mice, it is crucial to identify the T cells and cytokines that are present at the site of the lesions in vivo. The assessment of cytokine gene expression in the lymph nodes of mice infected with *Mycobacterium leprae* revealed that a Th_1 pattern of response corresponded with the induction of protective immunity (348), and a similar approach has been used to evaluate cytokine responses in the brains of CBA/CaH (type I) and BALB/c (type II) mice after sublethal intravenous infection with *C. albicans*.

These experiments failed to reveal any direct association between Th_1 and Th_2 patterns of cytokine response and the development of mild or severe lesions, in that cytokines typical of both Th subsets were detected in these animals. mRNAs for IFN-γ, IL-6, and TNF-α were readily demonstrated in infected mice of both strains but were present in significantly higher concentrations in the mice with severe lesions (15). The fungal burden and the severity of the tissue damage become established before the increase in concentrations of the cytokine mRNAs in the lesions, and yeast numbers peak at the same time and fall at the same rate in both mouse strains. Although the relative increase in the concentrations of IL-4 and IL-6 in the brains of CBA/CaH mice, especially at day 5 after infection (15), is consistent with a dominance of Th_2 cells in the host response, levels of the Th_1 cytokine IFN-γ were also consistently higher in this strain. The current concept that dominance of either the Th_1 or Th_2 subset determines whether a particular mouse strain exhibits resistance or susceptibility to *C. albicans* infection is clearly inadequate to explain these data. An alternative hypothesis, for which there is some precedent (246), is that infection is associated with successive waves of $CD4^+$ cells, the first producing IFN-γ and peaking at the time at which protective immunity is fully expressed, and the second secreting IL-4 and emerging at a time when the infection has been contained.

In this particular model, there was also evidence in both mouse strains of substantial activation or recruitment of $CD8^+$ cells. Screening of the brain cDNA for sequences coding for CD4 and CD8 proteins showed that the concentrations of CD4 remained relatively constant in both strains, whereas there was a 10-fold increase in CD8 mRNA levels in the brains of both BALB/c and CBA/CaH mice between days 3 and 5 after infection. $CD8^+$ lymphocytes with the ability to inhibit growth of *C. albicans* hyphae in vitro (40) can be induced from mouse lymph node (41) and spleen (42) cells by culture with rIL-2. Furthermore, after culture with *C. albicans* and IL-2 in vitro, $CD8^+$ lymphocytes kill *Candida*-infected neutrophils (290) and express MHC-restricted cytotoxicity against *Candida*-infected macrophage target cells (290, 291). $CD8^+$ lymphocytes secrete IFN-γ and other Th_1 cytokines (130) but under certain circumstances can also produce Th_2 cytokines such as IL-4 (67). In fact, recent studies have shown that IL-12 and IL-4 promote the development of type 1 and type 2 cytokine profiles, respectively, in both $CD4^+$ and $CD8^+$ lymphocytes (85).

The role of $CD8^+$ T cells in the pathogenesis of infection and resolution of tissue lesions in this model of systemic candidiasis remains to be explored. They may help to contain the infection by lysing infected PMNL, releasing the microbicidal agent calprotectin (218); they could contribute to recovery by the production of cytokines that activate phagocytic cells; or, alternatively, they may modulate the effector activity of $CD4^+$ Th cells, as has been demonstrated in *Schistosoma mansoni* infection (80). Differences in the efficiency of lysis of infected macrophages and PMNL by $CD8^+$ lymphocytes may also contribute to the genetically determined dichotomy in the severity of the lesions in different inbred strains of mice.

Discussion

It is pertinent here to recall the two themes central to the analysis of host resistance to *C. albicans* infection. These are (i) whether fundamentally different mechanisms are responsible for the clearance of systemic as distinct from mucosal infec-

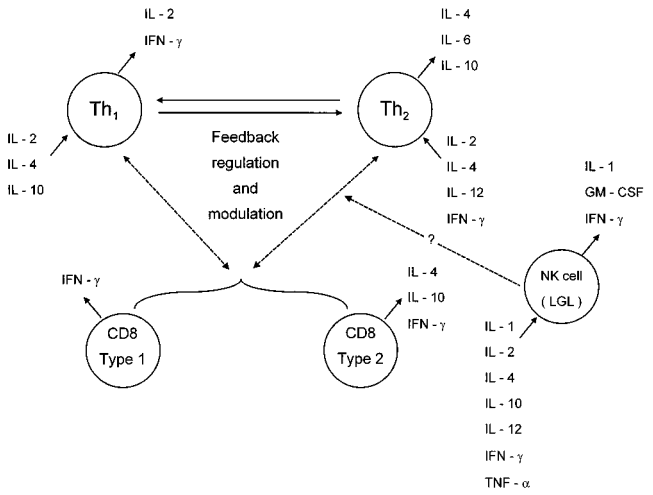

FIG. 2. Cytokine responsiveness of T lymphocytes and NK cells. Only those cytokines presently known to be relevant to *C. albicans* infection are shown. LGL, large granular lymphocyte. Short arrows pointing inward indicate cytokines that have an effect on the cell; those pointing outward indicate cytokines produced by the cell. The long arrows indicate regulatory pathways.

tions and (ii) at what level does the immune (?) defect that predisposes to recurrent mucosal infections occur.

Human studies have identified numerous effector pathways that result in *Candida* killing, but to date, no convincing link has been established between a particular clinical condition and any specific effector mechanism. This statement does not belittle the associations between systemic candidiasis and neutrophil deficiency or dysfunction and between chronic mucosal infections and abnormalities in the cell-mediated immune response; rather, it emphasizes that the effector mechanisms that have been characterized are not unique to either condition. In fact, cytokine-mediated augmentation of the phagocytic function of monocytes/macrophages and PMNL seems to provide a unifying thread between the systemic immune response and events occurring at the mucosal surface.

The recognition that cytokines represent not only a crucial link between T lymphocytes and phagocytic cells but an important regulatory circuit for the T cells themselves (Fig. 2) has led to a rapid expansion in our understanding of the mechanisms that determine the outcome of experimental *Candida* infections. A significant advance was the identification of the reciprocal effects on susceptibility and resistance of cytokines produced by the two subsets of $CD4^+$ Th lymphocytes. As human lymphocytes also show a functional division into Th_1 and Th_2 subsets (281), this finding raised the possibility of enhancing host resistance against systemic infection by redirection of the cytokine response by using monoclonal antibodies (220). Nevertheless, even though the Th_1/Th_2 paradigm has provided a useful theoretical framework for the analysis of the role of cytokines characteristic of these two T-cell subsets, it is becoming apparent that there are data that cannot be accommodated by this deterministic approach.

One problem that may be unique to the *Candida* model is the strain- and organ-dependent variability in the progression and outcome of infection. At least three different variables influence the host response to the yeast.

(i) Deficiency in C5. A deficiency in C5 would be expected to influence the host response throughout the body, but in fact, its effect is most clearly manifested in the kidney. Investigations of the role of complement C5 in *Candida* infection have been limited to its functions as an opsonin and chemoattractant, but complement components, C5a in particular, can also initiate or enhance the transcription and release of cytokines such as IL-1 and TNF (94) and thus exert a subtle influence on phagocytic and immune interactions. There is considerable evidence that differences in mortality in inbred strains are related to the ability of the kidney to constrain growth of the yeast, and in this specialized local environment, C5-mediated induction of cytokines may act to augment the candidacidal or candidastatic activities of resident phagocytes until these defenses are bolstered by recruitment of T lymphocytes and other inflammatory cells from the blood.

(ii) Strain-dependent differences in tissue damage. The severity of tissue damage in any particular inbred strain is under single-gene control but is unrelated to the presence or absence of C5 and is independent of the extent or duration of *Candida* colonization. The question of whether this dichotomy reflects differences in the intrinsic resistance of the tissue cells or in the properties of cells of the hematopoietic system has been addressed by constructing radiation chimeras between type I and type II mice. CBA/CaH mice, which exhibit severe lesions, were lethally irradiated and reconstituted with MHC-compatible bone marrow from BALB/k mice, which develop mild lesions. When these animals were subsequently challenged with a sublethal inoculum of *C. albicans*, lesions in the mice that had received the BALB/k bone marrow were substantially less severe than those in mice given syngeneic marrow (27). This result provides clear evidence that genetic differences in the extent of tissue damage in inbred mice are determined by intrinsic properties of the bone marrow precursor cells; however, the effect cannot as yet be attributed specifically either to the phagocytic cells or to the lymphocytes.

(iii) Organ-specific differences in fungal clearance. The total burden of yeasts in any particular organ in a given inbred strain may also determine the effectiveness of the host response in clearing the organism from infected tissues. This effect is most obvious in the brains of CBA/CaH and DBA/1 mice at 8 to 14 days after infection, but the mechanisms are at present unknown.

All of these factors operate within different time frames, and all add layers of complexity that need to be considered and evaluated in the interpretation of experimental studies of host responsiveness to *C. albicans.*

Comprehensive studies by Bistoni and his colleagues have confirmed the importance of IFN-γ in recovery from experimental systemic infection and demonstrated that preferential induction of IL-4 in certain mouse-yeast strain combinations downregulates these protective responses. While there is no doubt that these healer and nonhealer combinations display the appropriate Th_1 and Th_2 cytokine profiles, a pivotal question is how these become established in the host animals. This question is particularly cogent in view of the fact that the nature of the cytokine response can easily be modified by changing the route of antigen presentation (systemic or mucocutaneous) and/or the *Candida* strain used (virulent or avirulent), as well as by pretreatment with pharmacologically active agents such as streptozotocin. A case in point is the response of DBA/2 mice. In this strain, mortality is associated with the development of a Th_2 cytokine profile and survival is associated with a Th_1 profile. However, the high mortality can be attributed to the failure of the kidney to effectively constrain proliferation and growth of the yeast, whereas in sublethally infected mice, clearance and tissue damage in organs such as the brain are not substantially different from those in the more resistant strains. It follows that an improvement in survival in this strain may be achieved by manipulations that enhance the resistance of the kidney without necessarily affecting mecha-

nisms that determine clearance of the organisms from other organs and tissues.

Recent studies with transgenic mice have shown that in the absence of a strong phenotype-directing signal from the infectious agent, the genetic background in any given inbred strain can direct the development of the Th cells to a default phenotype (157), which in the DBA/2 strain is of the Th_2 type. By implication, when the mice are intravenously infected with a sublethal dose, either recovery takes place despite the onset of Th_2 cytokine production, as has been demonstrated to occur under certain conditions in murine cutaneous leishmaniasis (308), or the reduced infectious load may allow the development of a Th_1 cytokine response. A precedent for qualitatively different effects of quantitative changes in the antigenic stimulus can be seen in the induction of tolerance or rejection after challenge of female mice with large or small grafts of male skin (8). A reduction in the magnitude of the fungal burden may also account for the development of protective Th_1-type immunity after mucosal infection (44), under which circumstances the infectious pressure on the systemic immune response would be much reduced in comparison with infection via the intravenous route. Alternatively, interaction of the yeast with cells in the mucosa may lead to the local secretion of IFN-γ (122), which could direct the differentiation of peripheral $CD4^+$ T cells into the Th_1 cytokine pathway.

The relationship between the immune response at the mucosal surface or in a specific organ and that detected in the systemic circulation is still obscure. In mucosal infections with *C. albicans*, γ/δ T cells from all mouse strains may respond by the production of Th_1 cytokines, leading to the peripheral Th_1 response reported for most mucosal models. On the other hand, the type of Th cytokine profile elicited after systemic infection may not necessarily reflect events occurring within different organs or tissues, which may be influenced by site-specific factors such as the relative susceptibility of the tissue or the local fungal burden. Conversely, the extent to which the systemic cell-mediated immune response can protect from or ameliorate local or mucosal infections has yet to be clarified. Although there is little doubt that central defects render patients susceptible to severe mucosal infections, active immunization of mice confers only limited additional resistance.

This is not altogether unexpected, as there is likely to be a maximum rate at which the yeast can be eliminated from the mucosa. The limiting factors at this site are probably the number and rate of recruitment of phagocytic cells rather than the availability of T-cell-derived cytokines. As patients with chronic mucosal infections do not display any obvious defects in phagocytic cell production or function, the immunological abnormalities that lead to recurrent infection by the yeast are likely to lie in the regulatory circuits that govern cytokine production.

IMMUNOREGULATION IN *CANDIDA* INFECTION

Immune Modulation in Human Disease

Active suppression of cell-mediated immune responses has been associated with a number of fungal diseases (324), and infection with *C. albicans* induces both specific and nonspecific modulation of immune responsiveness. Polysaccharide antigens of *C. albicans* generate a complex series of interactions that result in the suppression of both T- and B-cell responses to the homologous antigen (257). These effects are mediated by an antigen-nonspecific inhibitory factor that is able to block the synthesis of both IL-2 and IFN-γ and the expression of the Tac antigen (the IL-2 receptor) by normal human T cells (196). This factor also inhibits antigen presentation and the production of IL-1 by human monocytes (197). Another *Candida* polysaccharide antigen has also been found to inhibit *Candida*-specific proliferation of normal human lymphocytes (129). This particular inhibitory activity—attributed to mannan—appears to require both $CD8^+$ and $CD8^-$ suppressor cells and results in the blockage of maturation of mannan-specific B cells (114). Induction of the T suppressor cells requires HLA-DQ-compatible macrophages; however, the interaction between T and B cells is unrestricted.

Immunoregulation in Murine Candidiasis

Phenomena comparable to those in humans have been reported for experimental animals. Systemic infection with *C. albicans* induces a transient loss of lymphocyte proliferative responsiveness to mitogens and to unrelated antigens (276). A similar impairment of T-cell but not B-cell responses to mitogens was obtained after treatment of mice with formalin-killed *C. albicans* (274). The effector cells, which were capable of suppressing the T-cell-dependent proliferative response of normal cells, were identified as a surface immunoglobulin-positive B lymphocytes. An antigen nonspecific suppressor population of Lyb-2.1^+ B cells could also be elicited in vitro by culturing normal splenocytes with formalin-killed *C. albicans* (88) and with antigens derived from it (86). The B suppressor cells required both T cells and accessory cells for their generation (87) and inhibited both primary and secondary T-cell-dependent antibody responses. However, they did not induce any detectable changes in either T-cell effector function or T-cell-independent antibody production (86). The same group has shown that culture of splenocytes with an extract of *Candida* cell walls generates a population of $CD4^+$ $CD8^-$ suppressor T cells that inhibit primary and secondary antibody responses to sheep erythrocytes (89).

Administration to mice of a *Candida* glycoprotein prior to a standardized immunization regimen results in suppression of DTH and lymphocyte proliferative responses to the homologous antigen and also diminishes the protective effects of immunization (71). *C. albicans* or its cell wall components can also induce different immunomodulatory phenomena, such as enhancement (91) or suppression (107) of antibody responses to third-party antigens. The most potent immunomodulatory activity has been associated with cell wall components of *C. albicans*, in particular with the mannans and mannoproteins. Mice injected with mannan at or near the time of immunization displayed enhanced antibody responses to both a T-cell-dependent (pneumococcal polysaccharide) and a T-cell-independent (sheep erythrocyte) antigen (112). However, when horse erythrocytes were used as a carrier for both the mannan and the pneumococcal polysaccharide, either enhancement or suppression of the antibody response was obtained. Although the nature of the response in vivo depended on the amount of mannan given with the immunizing antigen, the immunosuppressive and immunoenhancing components of the mannan were separable by chromatography.

A preparation of mannan was more efficient than an extract of cell wall glycoprotein in eliciting DTH responses from mice infected with viable blastospores, but the converse was found with lymphoproliferative responses (110). When mice were treated with mannan intravenously prior to immunization or, in the case of gastrointestinal colonization, between the priming and secondary inoculations, DTH reactions to mannan were significantly and specifically suppressed (110). The suppressor cells were characterized as Thy^+ $CD8^+$ and, on transfer to immunized recipient mice, suppressed DTH in a dose-dependent manner (134). A similar inhibition of DTH

TABLE 6. Summary of immunological reactivity in inbred and congenic resistant mice after primary and secondary challenges with *C. albicans*

Strain	MHC type	Lesion type	Primary response[a]	Secondary response[b]
CBA/CaH	*H-2*^{k}	Severe	Low	Low
BALB/c	*H-2*^{d}	Mild	High	High
BALB/k	*H-2*^{k}	Mild	Low	High

[a] Measured as the increase in cell numbers in the popliteal lymph nodes after infection via the footpad (9).

[b] Represents the magnitude of DTH and lymphocyte proliferative responses in mice that had recovered from a primary infection with *C. albicans* (11).

responses was demonstrated by passive transfer of T suppressor cells into cyclophosphamide-treated mice (336). The activity of the suppressor cells could be modulated so that they were unable to exert a suppressive effect on DTH responses after transfer into immunized mice if the donor mice were first treated with monophosphoryl lipid A, an experimental therapeutic agent derived from lipopolysaccharide (111). Nevertheless, even though treatment of mice with mannan induced profound inhibition of DTH responses, host resistance against systemic infection in these mice was unaffected (135); in fact, there was some evidence that mannan might actually have enhanced protection.

In another study, a protein with a relative molecular mass of 43 kDa, isolated from culture supernatants of a virulent but not an attenuated strain of *C. albicans*, was evaluated for its immunomodulatory effects in vivo (329). Treatment of mice with this protein increased the number of immunoglobulin-secreting plaque-forming cells in the spleen, specifically suppressed the primary immune response against sheep erythrocytes, and resulted in an increase in the number of yeasts in the kidney after intraperitoneal challenge. The immunosuppressive and B-cell mitogenic properties were directly correlated and showed an inverse relationship with the ability to induce susceptibility to *C. albicans* infection. Immunization of BALB/c mice with the protein partially neutralized the immunomodulatory effects (328) and fully protected the mice against the fungal infection.

Given that there is cogent evidence for a variety of immunomodulatory events associated with *C. albicans* infection, the point at issue is not the immunosuppressive potential of the yeast per se, but whether there exists a particular type of immune modulation that occurs within a genetically defined host environment and is associated with a differential susceptibility to infection.

Genetic regulation of immune responses. At present, the only documented differences in T-cell responses to primary *C. albicans* infection in type I and type II mouse strains are lower numbers of inflammatory cells but a higher proportion of T cells in the draining lymph node of a susceptible strain (CBA/CaH) after subcutaneous injection (9, 21). However, the evolution of the immune response, as reflected in assays of memory T-cell function, appears to be subject to different forms of regulation in type I and type II mice (Table 6). Both DTH and *Candida*-specific lymphoproliferative responses are significantly weaker in CBA/CaH than in BALB/c mice; however, in BALB/k mice, which have the same MHC haplotype as CBA/CaH mice, the magnitude of the memory responses is not significantly different from that in BALB/c mice (11). This differential responsiveness is governed not by MHC genes, as in the primary response, but by background or non-MHC genes. The weak T-cell memory responses in CBA/CaH mice are also associated with the occurrence in high frequency in this strain of a chronic osteomyelitis in the vicinity of the inner ear (25) that persists for at least 6 months.

A further analysis of the reactivity of activated spleen cells from CBA/CaH and BALB/c mice revealed a dichotomy between the presence of IFN-γ mRNA and protein production (14). mRNA for IFN-γ was demonstrated in primary and secondary cultures of both BALB/c and CBA/CaH lymphocytes as well as in immune cells cultured in the absence of *Candida* antigen, but biological assays revealed that on days 3 and 5 of culture, functional IFN-γ protein was secreted by lymphocytes from BALB/c but not CBA/CaH mice. Although the cell populations used in these experiments were heterogeneous and the readouts may have included extraneous responses, such as to fetal calf serum, the important point is that there is a functional difference between the reactivity of lymphocytes from BALB/c and CBA/CaH mice after culture in vitro with *Candida* antigens. Similar results were obtained independently by Romani et al. (288), who showed that even though IFN-γ mRNA was present in $CD4^+$ and $CD8^+$ lymphocytes from mice with either self-limiting or progressive infections, IFN-γ protein was produced only by lymphocytes from mice with self-limiting infections.

These data are consistent with the existence of qualitatively different regulatory mechanisms in strains of mice showing different patterns of tissue damage, and immunological cross-reactivity resulting in the generation of autoimmune responses has been proposed as a mechanism to explain both the generalized and specific unresponsiveness induced as a consequence of *C. albicans* infection (86) and the increased susceptibility to infection of patients with recurrent *Candida* vaginitis (20).

MOLECULAR MIMICRY

The concept of molecular mimicry in *C. albicans* infection is not new. Antibodies to single-stranded DNA were detected and found to correlate with disease activity in patients with chronic mucocutaneous candidiasis (366), and of more relevance to this discussion, patients with chronic vaginal candidiasis displayed high titers of antiovarian and antithymocyte antibodies (210). Significantly, the T-lymphocyte-specific antibodies in these patients were directed primarily against helper T cells. *C. albicans* also expresses cell surface receptors for the C3 fragment iC3b (141), which cross-react with some but not all monoclonal antibodies against epitopes of the human C3 receptor (216). However, although the fungal and human C3 receptors cross-react and bind identical ligands, they have been found to differ in structure (2). Other serological cross-reactivities have been demonstrated. A protein of *C. albicans* reacted with a monoclonal antibody (MY9) specific for a monocyte antigen (216), and yeast-phase cells express a protein that is antigenically and structurally related to CD11b/CD18, a member of the β2 family of integrins (145).

Heat shock protein 90 (hsp90) is an important regulatory protein in humans, and an antibody to a 47-kDa breakdown product of hsp90 is found in high frequency in the serum of patients recovering from disseminated candidiasis (215) and in the serum of patients with AIDS (213). These observations led to the suggestion that it may have a protective function, and further studies showed that an autoantibody to hsp90 could protect mice against systemic infection (214). This group has pointed out the apparent paradox that the recognition of self-reactive epitopes of heat shock proteins in *Candida* and other infections does not lead to autoimmune disorders (212) and suggested that such reactivity might take place within an "immunological homunculus" (82) consisting of preformed regulatory networks that channel the response into regulated path-

ways, preventing the development of autoimmune diseases. Nevertheless, genetic and other factors might conspire in some individuals to defeat the regulatory response, leading to autoimmunity.

In this context, the immune system has been shown to maintain a screening process in the periphery that can detect and induce tolerance in potentially autoreactive cytotoxic T lymphocytes (144). The process depends on the presence or absence of Th cells, in that if T help is available, the cytotoxic precursors are activated, whereas in the absence of help, the cells become tolerant. However, tolerance of $CD4^+$ cells apparently does not result in clonal deletion, as infection with the nematode *Nippostrongylus brasiliensis* is able to break T-cell tolerance established by injection of mice with *Staphylococcus* enterotoxin B (275), resulting in the clonal expansion in vivo of the $V\beta8^+$ $CD4^+$ cells. The persistence of potentially autoreactive T cells in the periphery was demonstrated many years ago by Blanden et al. (50), who also postulated that T-cell tolerance might be a quantitative phenomenon, related to the density of self-antigens to which the T cells were exposed during differentiation and selection in the thymus (49). A similar hypothesis has been invoked to explain the antigen-specific reactivity of self-tolerant unresponsive T cells (178). A necessary corollary is that an increase in the density of self-antigens should lead to the breaking of self-tolerance. This could be caused by, for example, cross-reactive antigens present on infectious agents or upregulated by infection.

Two different lines of evidence are relevant to *Candida* infection. First, human oral mucosa affected by *C. albicans* (170) and the lesions of angular cheilitis infected with *C. albicans* (245) displayed increased expression of HLA-DR by epithelial cells and keratinocytes, respectively. In both cases, an intense intraepithelial infiltration of T lymphocytes was observed, suggesting that the increased expression of the MHC class II antigens might be induced by T-cell-associated cytokines. Similar findings were obtained in mice (13) except that, in these experiments, a culture supernatant of *C. albicans* was effective in directly enhancing expression of MHC class II antigens in both LK cells (an antigen-presenting murine cell line) and thioglycolate-induced mouse macrophages. Thus, there is a quantitative increase in the expression of self-antigens during *Candida* infection, a prerequisite for breaking self-tolerance, as predicted by the theory.

Second, an analysis of the reactivity of activated spleen lymphocytes from CBA/CaH and BALB/c mice that had recovered from a primary systemic infection with *C. albicans* revealed that cells from mice of the former strain are capable of expressing autoreactivity, as measured by an increase in footpad thickness, after injection into the footpad of syngeneic normal mice in the absence of any further exposure to *Candida* antigens (12). This reactivity is mediated by $CD4^-$ $CD8^+$ T cells and is directed against antigens expressed in $(BALB/c \times CBA/CaH)F_1$ but not in $(BALB/c \times BALB/k)F_1$ hybrid mice. The activated cells did not display either specific nor nonspecific cytotoxicity, but as limiting-dilution analysis was not attempted, the existence of a minor population of cytotoxic cells could not be formally excluded. These data have been interpreted as favoring the existence in CBA/CaH but not in BALB/c mice of some form of immunoregulatory mechanism that has been activated as a consequence of molecular mimicry between *Candida* antigens and those of the host (24).

When viewed in this perspective, the various lines of evidence are consistent with the hypothesis that self-tolerance in type I mice is broken by infection. Regulatory mechanisms may then come into play to control the aberrant responses, so that the end result is not autoimmunity but the weakening of host defense mechanisms against the yeast. It is tempting to speculate that the weak DTH responses in these mice reflect the suppression of $CD4^+$ helper cells and that the effector cells suppressed by the response are $CD8^+$ cytotoxic cells specific for a determinant common to both yeast and host.

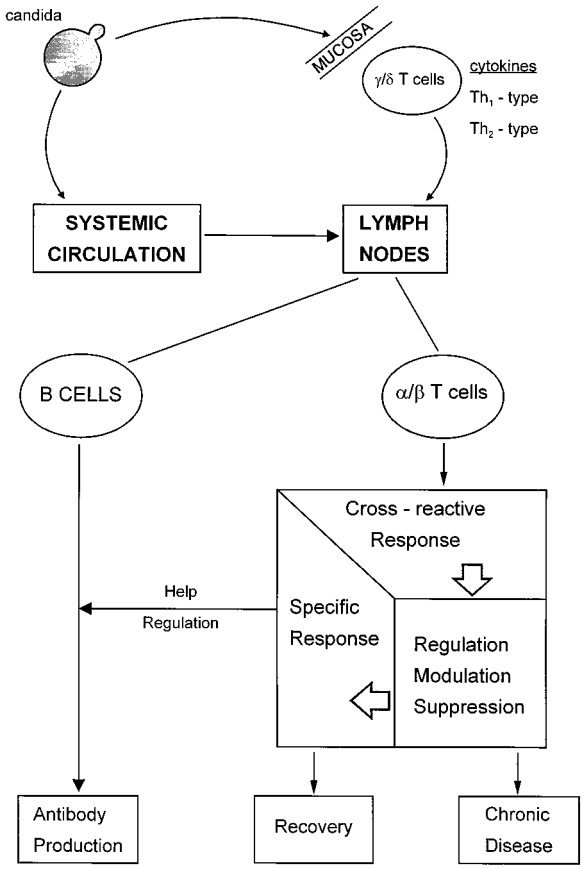

FIG. 3. Schematic illustrating alternative pathways in the host response to systemic and mucosal infection with *C. albicans.*

CONCLUSION

The general picture that is emerging from the mouse studies indicates that interactions at many different levels determine the outcome of mucosal or systemic challenge (Fig. 3). A striking feature of the relationship between *C. albicans* and the host is the subtle way in which responses vary under different conditions of systemic infection, especially as there is a narrow window for the establishment of disease before mortality supervenes. For reasons that are still unclear, some physiological quality of the mouse kidney determines the threshold at which animals will die, and stimulation of macrophage activation by nonspecific agents or through the Th_1-mediated cytokine pathway enhances the resistance of this organ and increases survival. When the infectious challenge is reduced to a sublethal dose, differences in the innate resistance of inbred strains become more obvious.

Altering the route via which *Candida* antigens are presented to the immune system, such as infection via the gastrointestinal tract rather than intravenously, can change the outcome of challenge from early mortality to systemic immunity and protection against reinfection. This effect could be mediated by qualitative differences in antigen processing or presentation or

by a direct effect of the antigenic load, but the pathways via which these effects are mediated remain to be defined. Clearly, these differences could be actively reinforced by the immunomodulating properties of mannan and other mannoprotein constituents of the yeast. These moieties may also interact with established immunoregulatory pathways that maintain self-tolerance to attenuate the specific response against the yeast in certain genetically susceptible hosts.

Although substantial progress has been made in the analysis of mechanisms of recovery from systemic candidiasis, the relationship between systemic and mucosal responses and the nature of the defect that leads to chronic disease remain major challenges for future research.

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