Chapter 17
Immunomodulators: Potential in Treatment of Systemic Fungal Infections

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Abstract Innate immunity mediates strong resistance to fungal pathogens and contributes to host defense against opportunistic fungal infections such as candidiasis, aspergillosis, and other rare infections. Immune factors such as cytokines and effector immune cells work synergistically with antifungal agents to restrict fungal growth. However, in immunocompromised hosts, the defectiveness of immune functions that should cooperate with antifungal drugs to clear the pathogens seems to be a critical factor that impedes the effectiveness of these drugs. The renovation or augmentation of immune responses is now considered as one of the foundations of effective antifungal therapy. Immunomodulation represents a novel approach to antimicrobial therapy that depends on boosting host immunity, rather than direct antimicrobial activity. Immunopotential therapy therefore offers a rational approach to the treatment of fungal infections, because it is intended to enhance immune functions in general. Major advances in the field of experimental immunology have provided insight into the important regulatory role of cytokines in both innate and adaptive immunity to fungal pathogens. Exploration has also begun with immunotherapy, with use of cytokines and immunomodulators alone or in combination with antifungal therapy. The administration of cytokines to patients, together with antifungal agents, offers promising immuno-therapeutic modalities

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for further research. The diverse array of natural, synthetic, and recombinant immunomodulators discussed in this chapter succinctly demonstrates the potential of these agents to stimulate host defense mechanisms for prophylaxis and treatment of various fungal infections.

17.1 Introduction

Fungal organisms are ubiquitous in nature. Although there are an estimated 250,000 fungal species, fewer than 150 have been described as human pathogens. Several reasons have been proposed for the increase in invasive fungal infections, including the use of antineoplastic and immunosuppressive agents, broad-spectrum antibiotics, and prosthetic devices and grafts, and more aggressive surgery. Patients with burns, neutropenia, human immunodeficiency virus (HIV) infection, and pancreatitis are also predisposed to fungal infection (Eggimann et al. 2003). Systemic fungal infections get into the blood stream and cause life-threatening infections, and opportunistic infections are mainly found in people with a weakened immune system that could be caused by any systemic or superficial infections. *Candida*, *Cryptococcus*, *Aspergillus* and pneumocystic fungi are some potent organisms involved in systemic fungal infections.

The AIDS epidemic is one of the most important factors which have contributed to the rising incidence of fungal diseases. Prior to the widespread usage of highly active antiretroviral therapy (HAART) in developed countries, up to 80% of HIV-infected persons developed mucosal candidiasis, while others developed cryptococcosis, histoplasmosis, or coccidioidomycosis during the course of their disease (Hajjeh and Warnock 2003). Analysis of U.S. National Center for Health Statistics (NCHS) death records showed that fungal infections were the seventh most common cause of infectious disease- related mortality in 1992, and that mycotic disease-related fatalities had increased more than threefold since 1980 (Pinner et al. 1996). Additional analysis revealed that candidiasis and aspergillosis were the two specific diseases that accounted for most of these deaths (McNeil et al. 2001).

Augmentation of the host defense response, improvement of the underlying disease, and resolution of the principal immune impairment are paramount for successful treatment of invasive mycoses in immuno-compromised patients. Systemic mycoses are associated with high morbidity and mortality rates despite advances in antifungal chemotherapy. Recent studies have shown that upregulating the host immune response by immunological adjuncts could be helpful. Immunostimulants enhance the overall immunity of the host, and present a nonspecific immune response against the microbial pathogens. They also work to heighten humoral and cellular immune responses, by either enhancing cytokine secretion, or by directly stimulating B- or T-lymphocytes. Immunotherapy offers many therapeutic advantages through the availability of a wide range of recombinant cytokines that exert their effects indirectly through leukocyte activation rather than directly on the fungus. Immunotherapy is designed to increase the number of phagocytic cells and shorten the duration of neutropenia, modulate the kinetics or actions of those cells at the site of infection, and/or activate the fungicidal activity of phagocytes to kill fungal cells more efficiently (Latge 1999; Roilides and Pizzo 1992). Administration of recombinant hematopoietic human cytokines, such as granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), interferon-γ, interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α), have been shown to decrease the duration of neutropenia, increase the microbicidal action of neutrophils, monocytes, and macrophages, and reduce the duration of cytotoxic chemotherapy in systemic mycoses (Roilides and Pizzo 1992; Kullberg 1997). In this chapter we will deal with immunomodulators (synthetic and natural).

17.2 Immunomodulators

The immune system can be manipulated specifically by vaccination or nonspecifically by immunomodulation (Masihi 1994a, b, 1996, 1997; Masihi and Lange 1988, 1990). Immunomodulators are biological or synthetic substances capable of altering the immune response by augmenting or reducing any of the components of the immune system including both innate and adaptive arms of the immune response. Immunomodulators are usually products of the immune system (Committee on New Directions in the Study of Antimicrobial Therapeutics 2006). Basically, immunomodulators are agents that alter the immune response by suppression (immunosuppressive) or enhancement (immunostimulant) (Saunders Comprehensive Veterinary Dictionary 2007). Synonymous terms for immunomodulators include biological response modifiers, immune-augmentors, or immunorestoratives. Microbial products, drugs of natural and synthetic origin, and proteins derived from the immune system represent some of the immunomodulators that are currently in use.

Immunomodulators correct weak immune systems and temper immune systems that are overactive, but they do not boost the immune system the way immune stimulants such as *Echinacea* do. Immunomodulators are recommended for people with auto-immune diseases and they are widely used in chronic illness to restore immune system health in people who have been on lengthy courses of antibiotics or antiviral therapies. Certain antibiotics can, in addition to their antibacterial properties, also modulate the immune response (Labro 1998; Stevens 1996). Some immunomodulators are naturally present in the body, and certain of these are available in pharmacologic preparations.

Approaches to immunomodulation can be divided into those that are specific to pathogens (pathogen-specific) and those that are not (nonspecific). Specific immunomodulators are administered together with antigen, for instance in vaccines, where they are known as immunological adjuvants, and boost the immune response to the vaccine candidates (Bomford 1988). In principle it is possible to imagine a specific immunosuppressant which, when given together with antigen, would induce a state of specific nonresponsiveness or tolerance to the antigen.

Pathogen-specific immunomodulators include antibody reagents and vaccines. With the exception of the rabies and varicella zoster vaccines, currently licensed vaccines are administered to prevent acute infectious diseases rather than for therapy and are not discussed further here (Pirofski and Casadevall 2006). Nonspecific immunostimulants are given on their own in order to elicit a generalized state of resistance to pathogens which in many cases is believed to depend on the activation of macrophages (Bomford 1988). Nonspecific immunosuppressants reduce the capacity of the immune system to respond to antigens either by the blunderbuss approach of killing dividing cells with cytotoxic drugs, or by interfering with the function of cells of the immune system in a more selective way. Nonspecific immunomodulators include cytokines, antimicrobial peptides, certain antimicrobial drugs, and microbes such as probiotics. At present, clinical experience with nonspecific immunomodulators as antimicrobial tools has been predominantly limited to cytokines.

Immunostimulatory agents do not directly affect immune memory cells, as activation and differentiation of memory cells require precise cell–cell and MHC–antigen interactions. However, they are specific in that immunostimulants enhance particular immune responses to combat specific pathogens. Immunostimulating activities may be divided into those that (1) enhance phagocytic activities, and (2) effect cell-mediated and humoral immunity (Tan and Vanitha 2004).The modulation of the immune response has a number of important implications. For example, the adjuvant action of the cytokine, lymphokine, hormone, or growth factor can increase the concentration of protective antibodies produced against the antigenic portion of the conjugate in the vaccinated organism. Likewise, antibody production against antigens coadministered with the conjugate can be increased. As a result, effective (i.e., protective) vaccination can be achieved with a smaller quantity of conjugated antigen and/or co-administered antigen than would be normally required. This reduction in the required amount of conjugated antigen and co-administered antigen may lead to more widespread use of vaccines which are difficult or costly to prepare or which are weakly immunogenic. This is especially true in the developing nations that face such epidemics as malaria and cholera, with very limited health care budgets. It may also provide for safer vaccination when the antigen is toxic at the concentration normally required for effective immunization. By reducing the amount of antigen, the risk of toxicity may be reduced.

17.3 Combinational Trends of Antifungals with Immunomodulators

Since fungal infections occur mainly in immunosuppressed patients, it is reasoned that adding an immunomodulator or stimulator to an antifungal agent may improve the chance of a successful outcome. Consequently, researchers have sought to determine the effects of adding immune factors (e.g., granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor) or effector cells [e.g., primarily amacrophages, polymorphonuclear neutrophils (PMNs), and monocytes] to antifungal drug regimens in an attempt to manipulate both innate and adaptive host defenses. Effective antifungal agents may act in collaboration with host effector cells at various intracellular and extracellular locations, or during different postinfection intervals corresponding to different phases and mechanisms of host defense response (Chiller et al. 2001, 2002; Stevens 1998). Overall, various antifungals combined with immunomodulators against candidiasis have been shown to be generally more effective than monotherapy (Chiller et al. 2002; Coste et al. 2002; Kuhara et al. 2000; Mencacci et al. 2000a; Vora et al. 1998a, b). Adjunctive immunotherapy using antibody-based therapies has been investigated for *Cryptococcus neoformans* and *A. fumigatus* infections and generally shows enhanced activity with combination therapies (Clemons and Stevens 2001; Feldmesser et al. 1996; Mukherjee et al. 1994, 1995; Nassar et al. 1995; Roilides et al. 2002; Vora et al. 1998a, b). Different studies in this field have been summarized in previous reviews (Stevens 1998; Stevens et al. 1998, 2000).

Some case reports have been described supporting the notion that immunomodulators can influence the efficacy of antifungal agents (Chiller et al. 2001; Ellis et al. 2002). These studies suggested that immunomodulators may be acting via neutrophils (Th_1 response) or monocytes (inducing tumor necrosis factor and macrophage inflammatory protein 1). In separate studies, voriconazole, posaconazole, and itraconazole enhanced the antifungal functions of human PMNs against hyphae of *S. prolificans* and *S. apiospermium* (Gil-Lamaignere et al. 2002b). Similarly, AmB lipid complex plus PMN displayed a significant additive effect against both *Scedosporium* species (22% for *S. prolificans* and 81% for *S. apiospermum*) (Gil-Lamaignere et al. 2002a). Efficacies of L-AmB plus granulocyte colony-stimulating factor have also been demonstrated *in vivo* by using an immunosuppressed murine model of disseminated *Scedosporium* infection (Ortoneda et al. 2002). Recently, Steinbach et al. (Steinbach et al. 2004) used disk diffusion, microdilution checkerboard, and gross and microscopic morphological analyses to demonstrate that a combination of the immunosuppressants cyclosporine or tacrolimus (FK506) with CAS exhibits a positive interaction against *A. fumigatus*.

In candidemia, fluconazole remains the drug of choice in neutropenic and nonneutropenic patients in whom *C. krusei* is unlikely and who have received no prior treatment with fluconazole. However, AmB is the agent of choice when infection is due to a fluconazole-resistant organism or *C. krusei*, or in patients who develop candidemia while on fluconazole therapy (Sheehan et al. 1999). Combination therapy of various drugs is also recommended, for example fluconazole is the drug of choice for the treatment of cryptococcal meningitis and is also the agent of choice for prophylaxis against cryptococcal meningitis in AIDS patients following initial therapy with AmB with or without flucytosine. High-dose fluconazole in combination with amphotericin B and flucytosine is the current treatment approach for the disseminated trichosporonosis in an immunocompromised host (Groll and Walsh 1999).

Antifungal drugs such as fluconazole and amphotericin B have shown broad immunomodulatory properties (Yamaguchi et al. 1993). Cytokines, effector cells, and antifungals seem to work synergistically to restrict fungal growth in immunocompetent persons (Stevens 1998). In immunocompromised hosts, the lack of effector functions that cooperate with antifungal drugs to clear the pathogens seems to be a crucial factor in impeding the effectiveness of the drug (Stevens 1998; Roilides et al. 1998a). Antifungal chemotherapy in conjunction with immunostimulatory molecules such as IL-12, IFN-γ and GM-CSF has been found to show enhanced efficacy against many fungal pathogens (Casadevall and Pirofski 2001). In *in vivo* murine models of *S. prolificans* infection, combined administration of liposomal amphotericin B and G-CSF has been reported to be effective (Ortoneda et al. 2002).

Taken together, these studies show that combining an antifungal agent with concomitant improvement of host immune response through the use of an immunostimulator is a promising area that needs to be investigated through experimental animal systems and clinical trials. A clear demonstration of the clinical relevance of this approach is the decrease in the incidence of esophageal candidiasis in the HIV/AIDS setting, resulting from host immune reconstitution brought about by the use of HAART (Ghannoum 2001). Although combining an antifungal with another therapeutic class has shown promise, more studies are needed to determine whether these combinations have widespread clinical relevance.

17.4 Tuftsin: A Highly Effective Immunomodulator

Tuftsin ($C_{21}H_{40}N_8O_6$) is a tetrapeptide (Thr^{289}-Lys^{290}-Pro^{291}-Arg^{292}) produced by enzymatic cleavage of the Fc-domain of the heavy chain of immunoglobulin G. It is produced primarily in the spleen. Tuftsin was first identified in 1970 by scientists Najjar and Nishioka. It was named after Tufts University where the peptide was discovered. Tuftsin binds to specific receptors on the surface of macrophages and polymorphonuclear leukocytes, stimulating their migration, phagocytic, bactericidal, and tumoricidal activity. It also influences antibody formation. Tuftsin deficiency, either hereditary or following splenectomy, results in increased susceptibility to certain infections, for example those caused by capsulated organisms such as *H. influenzae*, pneumococci, meningococci, and salmonella (Constantopoulos et al. 1972, 1973; Najjar 1981). Tuftsin has been chemically synthesized and it is considered for use in immunotherapy (Fig. 17.1).

Tuftsin, due to its hydrophilic character, cannot be grafted on the surface of liposomes without being attached to a sufficiently long hydrophobic anchor. Structure–function studies of this tetrapeptide indicate that its binding and subsequent activation of the mononuclear phagocyte system (MPS) is dependent upon rather strict conservation of its molecular structure. Thus, modifications of the peptide at its N-terminus or within the chain lead to a significant reduction or even loss of its biological activity (Fridkin and Gottlieb 1981). However, the activity is largely

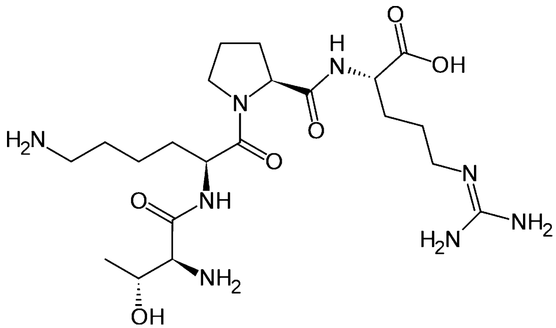

Fig. 17.1 Molecular skeleton of tuftsin [Pubchem (ID 24780)]

$$\text{Thr-Lys-Pro-Arg-NH-}(CH_2)_2\text{-NH-}\underset{\displaystyle \overset{\|}{O}}{C}\text{-}C_{15}H_{31}$$

Fig. 17.2 Structure of palmitoyl tuftsin (I)

retained if modifications are restricted only to the C-terminus (Gottlieb et al. 1982). All the modifications are therefore limited to the carboxyl group of the Arg residue. Direct attachment of a fatty acyl group to the Arg residue, without any spacer arm, leads to modified tuftsin, which does not allow formation of liposomes, presumably due to perturbation of the phospholipid polar head group packing by the bulky Arg residue (Singhal et al. 1984). This problem is, however, circumvented by introducing an ethylenediamine spacer arm between the Arg residue and the hydrophobic anchor (Fig. 17.2).

Liposomes containing palmitoyl tuftsin (I) specifically recognize macrophages and PMN leukocytes (Singhal et al. 1984). Treatment of macrophages with these liposomes considerably increases their respiratory burst activity (Singh et al. 1992). Pretreatment of animals with tuftsin-bearing liposomes enables them to resist malaria (Gupta et al. 1986); leishmania (Guru et al. 1989); antifungal (Owais et al. 1993), and antifilarial (Owais et al. 2003) drugs in liposomes containing palmitoyl tuftsin is shown to increase the therapeutic efficacy of drugs against these infections. Gupta and Haq (2005) described procedures for preparation of I as well as the liposomes that contain I in their bilayers, entrapment of various drugs in these liposomes, and their delivery to experimental animals with infected *L. donovani*, *M. tuberculosis*, or *Aspergillus*.

Immunomodulator-based therapy seems likely to be more beneficial for treatment of fungal infectious diseases. The co-administration of tuftsin increased the efficiency of liposomised-polyene antibiotics (nystatin and amphotericin B) against experimental murine candidiasis in immunocompromised Balb/c mice. Pretreatment with liposomised tuftsin prior to *C. albicans* infection clearly enhanced protection against candidiasis, suggesting a prophylactic role of tuftsin in normal and temporarily neutropenic mice (Khan et al. 2004). One of the pioneer researches by Khan et al. (2005a) showed the immunopotentiating efficacy of tuftsin against experimental murine aspergillosis in both normal and immunodebilitant BALB/c mice. They found that co-administration of the immunomodulator tuftsin and liposomised-amphotericin B was highly effective in the treatment of systemic infection of *A. fumigatus* in both cases, resulting in successful elimination of fungal pathogen (Khan et al. 2005b). In another study, Khan and Owais (2005) evaluated the combination of liposomal amphotericin B (lip-Amp B) and immunomodulator tuftsin to cure *C. neoformans* infection in BALB/c mice. Pretreatment of mice with liposomal tuftsin before challenging them with the *C. neoformans* infection resulted in 100% survival of the treated animals followed by treatment with lip-Amp B. In another set of experiments, they conducted the same study in leukopenic mice and found that incorporation of tuftsin in liposomes resulted in increased anticryptococcal activity of liposomal amphotericin B compared with amphotericin B deoxycholate and conventional liposomal amphotericin B formulations (Khan et al. 2005b).

Interestingly, tuftsin also increased the stability of liposomal amphotericin B. Our group has also demonstrated that co-administration of immunomodulator tuftsin along with liposomal formulations of amphotericin B successfully minimizes toxicity, as well as other side-effects of the drug (Masood and Owais 2006). The pharmacokinetics of amphotericin B in *Candida albicans*-infected mice treated with conventional and tuftsin-loaded amphotericin B liposomes was evaluated and was found to exhibit superior efficacy, safety, and favorable pharmacodynamics, therefore suggesting their potential therapeutic value in the management of fungal infections (Khan and Owais 2006).

Tuftsin-bearing nystatin was found to be effective in eliminating a strain of *C. albicans* less susceptible to amphotericin B (*C. albicans* JMCR) in Balb/c mice, but it may not be recommended due to toxicity constraints (Khan et al. 2003). Treatment with tuftsin-loaded nystatin liposomes was most effective in eliminating fungal burden from lung tissues of infected mice compared to those treated with free nystatin or nystatin liposomes without tuftsin. (Khan et al. 2006).

17.5 Cytokines as Nonspecific Immunomodulators

Invasive fungal infections (IFI) constitute a major threat for immunocompromised hosts. Particularly susceptible to IFI are patients with hematological malignancies and either disease- or treatment-related immunosuppression, including acute leukemia, especially acute myeloid leukemia (AML), chronic leukemia, lymphomas, and multiple myeloma, and recipients of allogeneic hematopoietic stem cell transplants (HSCT). The increased susceptibility of these patients to IFI has been attributed to several factors, including the underlying hematological malignancy, prolonged neutropenia, and impairment of host defense mechanisms because of intensive cytotoxic therapy or corticosteroid use, ablative radiotherapy, severe gastro-intestinal mucosal damage, delayed engraftment or graft-versus-host disease (GVHD) (Viscoli et al. 1999; Pagano et al. 2001; Martino and Subira 2002).

Preclinical studies have convincingly demonstrated that immunomodulation with cytokines can enhance the antifungal activity of neutrophils and monocytes/macrophages as well as upregulate protective T-helper type 1 adaptive immune responses. There is some evidence that Th_1 immune responses may be necessary for the optimal control of fungal infections (Brieland et al. 2001; Centeno-Lima et al. 2002). In this regard, immune interventions to polarize the immune response toward a Th_1 type may be beneficial. Evidence of Th_1/Th_2 dysimmunoregulation in hepatosplenic candidiasis (Roilides et al. 1998b) and invasive aspergillosis (Roilides et al. 2001), characterized by increased circulating levels of IL-10, has been demonstrated in humans. The utility of adjunctive therapy using immune modulating agents, such as hematopoietic growth factors (HGFs) or granulocyte transfusions, continues to be a matter of debate. No definitive randomized studies have been performed. Up to now, studies have only justified the safety of immunomodulating therapy, with anecdotes suggesting efficacy.

17.5.1 Hematopoietic Growth Factors (HGF)

HGF are able to augment the number of circulating phagocytes and their precursors. Of the HGFs, Granulocyte colony-stimulating factor (G-CSF) stimulates the proliferation and differentiation of myeloid progenitor cells to PMN leucocytes. Apart from increasing the number of mature neutrophils, G-CSF also enhances their phagocytic activity *in vitro* against a variety of pathogenic fungi, including *Candida*, *Aspergillus*, and *Fusarium* spp. (Roilides et al. 1993; Natarajan et al. 1997; Gaviria et al. 1999). Furthermore, ex vivo incubation with G-CSF was shown to enhance the impaired respiratory burst of neutrophils derived from transplant recipients against *Candida* and *Cryptococcus* yeasts as well as *Aspergillus* and *Rhizopus* conidia (Pursell et al. 2003). Recent studies, however, have demonstrated an additional important role of G-CSF in the regulation of adaptive T helper-cell responses. In particular, G-CSF promotes the “nonprotective” Th_2 responses through functional G-CSF receptors in T cells and monocytes (Boneberg et al. 2000; Franzke et al. 2003). In ex vivo lipopolysaccharide-stimulated whole blood, G-CSF treatment attenuated the release of IL-12, IL-1β, IFN-γ and TNF-α (Boneberg et al. 2000).

Macrophage colony-stimulating factor (M-CSF) accelerates the proliferation and differentiation of monocyte myeloid progenitors, and enhances chemotaxis, phagocytosis, and secondary cytokine production in mature monocytes and macrophages (Nemunaitis 1998). Incubation of macrophages with M-CSF enhances the killing of *Candida* spp. and *Cryptococcus* spp. Treatment of chronic disseminated candidiasis in rats with M-CSF has been shown to reduce the outgrowth of *C. albicans*. It enhances monocyte/macrophage antifungal activity against *C. albicans*, *A. fumigatus*, *H. capsulatum*, and *T. asahii* (Khemani et al. 1995; Roilides et al. 1995b, Sasaki et al. 2000; Gonzalez et al. 2001). These *in vitro* data were in agreement with animal studies of invasive candidiasis, aspergillosis, and trichosporonosis, where

M-CSF treatment was associated with improved survival and reduced fungal burden (Cenci et al. 1991; Sasaki et al. 2000; Gonzalez et al. 2001).

Granulocyte–macrophage colony-stimulating factor (GM-CSF) accelerates haemopoiesis in the early steps of differentiation of myeloid cells, resulting in increased production of neutrophils, monocytes, and eosinophils. It also stimulates a variety of functional activities in these cells, including phagocytosis of fungal organisms by neutrophils or monocytes/macrophages (Armitage 1998). GM-CSF enhances TLR2 expression (important for response to yeast zymosan) and TLR2-mediated IL-8 responses in neutrophils (Kurt-Jones et al. 2002). It also enhances the expression of Dectin-1, which is the major receptor for the β-glucans of fungal cell wall, in murine macrophages (Willment et al. 2003).

17.5.2 Anti-inflammatory Cytokines

IFN-γ produced by T and Natural Killer (NK) cells is a key cytokine both in the innate and adaptive immune response to IFI. It stimulates migration, adherence, and antifungal activity of neutrophils and/or macrophages against *C. albicans*, *A. fumigatus*, *F. solani*, *T. beigelii*, and *P. marneffei* (Lyman et al. 1994; Gaviria et al. 1999; Kudeken et al. 1999; Mencacci et al. 2000b) The observed augmentation of antifungal activity by IFN-γ *in vitro* was in agreement with results of animal studies of experimental candidiasis and aspergillosis (Mencacci et al. 2000b). IL-12 is required for the development of protective Th1 responses against fungal infections. This important regulatory role is partly mediated by IL-12 induction of IFN-γ and IL-18 production (Romani et al. 1997; Kawakami et al. 2000a) and has been demonstrated in animal models of invasive candidiasis, aspergillosis, cryptococcosis, and paracoccidiosis (Romani et al. 1994; Cenci et al. 1998; Decken et al. 1998; Brieland et al. 2001; Arruda et al. 2002). Interferon gamma may be superior at enhancing the antifungal activity of phagocytes (Roilides et al. 1995a; Gaviria et al. 1999). The efficacy of adjunctive interferon-gamma 1b (IFN-γ 1b) with amphotericin B was studied in a Phase II, double-blind placebo-controlled trial for AIDS-associated cryptococcal meningitis (Pappas et al. 2004). The rationale for interferon therapy for cryptococcosis has a strong basis in preclinical studies in mice (Lutz et al. 2000) and in a human study showing an association between cerebrospinal fluid levels of IFN-γ and treatment in HIV-infected patients with cryptococcal meningitis (Siddiqui et al. 2005).

Two other cytokines, IL-15 and IL-18, play a role in the protective adaptive or innate immune response against IFI. IL-18 is involved in the development of Th_1 response through its stimulatory effect on the production of IFN-γ (Stuyt et al. 2002). It was shown to protect against *C. albicans* or *C. neoformans* infection in animal models and to restore defective Th_1 immunity to *C. albicans* in caspase 1-deficient mice (Kawakami et al. 2000b; Mencacci et al. 2000c; Stuyt et al. 2004). IL-15 is involved in the innate immunity against fungal infections by enhancing the antifungal activity of polymorphonuclear or monocyte cells against *C. albicans* and

A. fumigatus (Musso et al. 1998; Vazquez et al. 1998; Winn et al. 2003). An additional role of IL-15 in NK cell activation has recently been demonstrated (Tran et al. 2003).

17.5.3 Pro-inflammatory Cytokines

IL-4 is one of the cytokines associated with the development of Th2 response against fungal pathogens. It also suppresses phagocytic activity of monocytes/macrophages against C. albicans (Cenci et al. 1993; Roilides et al. 1997). IL-4 was shown to impair host resistance to *A. fumigatus*, *H. capsulatum*, *C. neoformans* and Paracoccidioides braziliensis in animal models (Cenci et al. 1999; Kawakami et al. 1999a; Gildea et al. 2003; Pina et al. 2004). The suppressive effect of IL-10 on the innate and protective Th_1 antifungal responses was demonstrated in mouse models of invasive candidiasis, aspergillosis, and histoplasmosis (Tonnetti et al. 1995; Del Sero et al. 1999; Vazquez-Torres et al. 1999; Clemons et al. 2000; Deepe and Gibbons 2003). Furthermore, it was recently shown that IL-10 produced from dendritic cells is required for activation of $CD4^+$ $CD25^+$ T_{reg} cells (Montagnoli et al. 2002). Taken together, the data presented for IL-10, IL-4 and IL-12 suggest that, for optimal development and maintenance of protective responses against fungal pathogens, a finely regulated balance of these directive cytokines, rather than the relative absence of opposing cytokines, appears to be required (Mencacci et al. 2000b).

TNF-α is a pro-inflammatory cytokine necessary for the development of effective innate and adaptive immunity to fungal infections. It stimulates antifungal effector functions of neutrophils and/or macrophages against *C. albicans*, *A. fumigatus*, and *C. neoformans* (Roilides et al. 1998c; Kawakami et al. 1999b; Mencacci et al. 2000b; Netea et al. 2004). It also induces a number of other cytokines, including IFN-γ IL-1, IL-6 and IL-12 (Netea et al. 2004). The role of TNF-α in the development of protective Th_1 responses was demonstrated in animal models of candidiasis, aspergillosis, and cryptococcosis (Mencacci et al. 2000b; Bauman et al. 2003).

17.5.4 Cytokine Therapy in Neutropenic Hosts

During the past two decades, invasive fungal infections have emerged as a major threat to immunocompromised hosts. Patients with neoplastic diseases are at significant risk for such infections as a result of their underlying illness and its therapy. *Aspergillus*, *Candida*, *Cryptococcus*, and emerging pathogens, such as the zygomycetes, dark walled fungi, *Trichosporon*, and *Fusarium*, are largely opportunists, causing infection when host defenses are breached. The immune response varies with respect to the fungal species and morphotype encountered. The risk for particular infections differs, depending upon which aspect of immunity is impaired.

Shortening the duration of neutropenia by use of recombinant human cytokines permits more intensive cytotoxic chemotherapy, thereby decreasing the duration and frequency of invasive fungal infections.

G-CSF and GM-CSF are used frequently in patients who are neutropenic and have invasive fungal infections. Adjunctive immunotherapy may be especially important for treatment of mould infections characterized by a large circulating fungal burden and relative resistance to antifungal drugs, as with disseminated fusariosis. In addition, other reports emphasize that outcomes of therapy for zygomycosis are improved with rapid resolution of neutropenia (Kontoyiannis et al. 2000). The potential utility of neutrophil transfusions as adjunctive therapy has been rejuvenated with the development of G-CSF-primed community donor transfusions. Studies evaluating the safety and efficacy of such transfusions, and the use of interferon-gamma for adjunctive therapy of aspergillosis in neutropenic patients are either ongoing or in development.

Reconstitution of the effector cells both numerically and functionally by treatment with leucocyte transfusions (WBCTx) from donors treated with G-CSF has also been attempted. Some patients with persistent neutropenia and infections refractory to conventional antifungal antibiotics appear to respond to adjuvant WBCTx (Roilides et al. 1998a). But, WBCTx may cause severe adverse reactions in the recipient. Therefore, careful selection of the donor, collection technique, and recipient are important. Recent studies with GM-CSF suggest that this recombinant cytokine may be active as an adjunctive therapy in the management of invasive fungal infections in cancer patients. The American Society for Clinical Oncology recently provided guidelines for patients receiving G-CSF and GM-CSF. These cytokines should be used when the expected incidence of febrile neutropenia is >40% in order to avoid infectious complications and to maintain dose intensity in subsequent treatment cycles. These cytokines were also recommended, in combination with autologous progenitor cells transplantation, after high dose chemotherapy.

Recovery from neutropenia is considered critical in cases of *S. prolificans* infection because this infection has a poor outcome (mortality rate approaching 100%) in persistently immunosuppressed patients despite aggressive systemic antifungal therapy (Barbaric and Shaw 2001; Revankar et al. 2002). Early detection, surgical removal of infected tissue (if possible), and immunorestoration appear to be the major means of halting progression of this devastating infection (Rippon 1988; Perfect and Schell 1996). *In vitro*, interferon-γ and GM-CSF can enhance neutrophil superoxide production, increasing the damage of *S. prolificans* hyphae by neutrophils and enhancing the fungicidal activity of macrophages-monocytes, thereby showing a positive immunomodulatory effect against this hyalohyphomycete (Groll and Walsh 2001; Gil-Lamaignere et al. 2001). Both G-CSF and GM-CSF accelerate myelopoiesis and decrease the duration of neutropenia, but they are different cytokines with different targets and immunomodulatory effects. Both *in vitro* and *ex vivo*, G-CSF, GM-CSF, and M-CSF have been shown to increase the fungicidal action of phagocytes against *Candida* and *Aspergillus* in a variety of experimental systems (Roilides et al. 1995a, b).

17.5.5 Recombinant Cytokines

Various biopotent molecules have been studied for their potential to modulate and restore impaired immune functions required to resist fungal infections. Recombinant cytokines and cationic peptides are two classes of low-molecular-weight compounds that have shown promise in this area of research. These include recombinant human cytokines including granulocyte colony-stimulating factor (rHuG-CSF), recombinant human macrophage colony-stimulating factor (rHuM-CSF), interferons, etc., some of which have shown encouraging results (Shukla et al. 1992). The addition of cytokines and other immunomodulatory approaches to antifungal therapy of cryptococcosis are actively being explored (Casadevall and Pirofski 2001; Lutz et al. 2000; Clemons et al. 2001). Studies are currently ongoing in animal models and phase I/II human trials with recombinant human gene product interferon (IFN)-γ, and monoclonal antibodies directed against cryptococcal capsular polysaccharide (Lutz et al. 2000; Clemons et al. 2001; Pappas et al. 2001; Larsen et al. 2002).

17.6 Plant Components as Immunomodulatory Agents

The use of plant products as immunostimulants has a traditional history. However, the isolation of the active principals involved did not gain momentum till the nineteenth century. Plants synthesize chemicals as part of their defense against pathogens. Many such compounds occur in nature as anti-feedant and anti-infectant chemicals, and are found effective against microbes.

> "Four vegetables are indispensable for the well being of man: Wheat, the Grape, the Olive and the Aloe. The first nourishes him, the second raises his spirit, the third brings him harmony and the fourth cures him".
>
> Christopher Columbus

Among the natural (plant) products studied in Central Drug Research Institute (CDRI) for immune modulating activity, iridoid glucosides from *Nyctanthes arbor-tristis* showed a promising immunomodulatory effect against systemic candidiasis in mouse (Khan et al. 1995). The ethanol (50%) extracts of seeds, roots, and flowers of *N. arbortristis* (arbortristosides A and C) showed immune stimulant activity based on enhanced haemagglutinating antibody (HA) titre, plague forming cells (PFC) counts, delayed type hypersensitivity (DTH), and macrophage migration inhibition (MMI). The immune stimulant effect of seed was, however, more significant in ethanol extract compared to *n*-butanol fraction of all the plant parts. The protective effect of these extracts/fractions was found to be possibly due to immune stimulatory activity of arbor-tristoside A and C elicited by significant ($<$0.001) increase in humoral and DTH response to sheep red blood cells (SRBCs) and MMI in Balb/c mouse (Khan et al. 1995).

Aloe vera, also known as the medicinal aloe, is a species of succulent plant that probably originated in Northern Africa. *Aloe vera* has been used as an immunostimulant having antibacterial and antifungal activities. *Aloe vera* extracts have been shown to inhibit the growth of fungi that cause tinea (Shamim et al. 2004). Topical application of *Aloe vera* may be effective for genital herpes and psoriasis (Vogler and Ernst 1999). However, it is not effective for the prevention of radiation-induced injuries, nor does it offer protection from sunburn or suntan (Feily and Namazi 2004). In a double-blind clinical trial the group using an *Aloe vera* containing dentifrice and the group using a fluoridated dentifrice both demonstrated a statistically significant reduction of gingivitis and plaque (de Oliveira et al. 2008).

Acemannan, the major fraction of aloe polysaccharides, has been extensively studied for immunomodulatory effects. Reports showed that these β (1,4)-linked acetylated mannans are able to increase phagocytic activities (Egger et al. 1996; Jae et al. 2001). CARN 750, an acemannan, stimulated leukocytes and lymphocytes in a dose-dependant manner, as well as triggered the release of IL-1, IL-6 and TNF-α. Administrations of CARN 750 also showed a positive influence on lymphocyte proliferation in the spleen and bone marrow (Egger et al. 1996), both of which are essential lymphoid organs that produce and differentiate lymphocytes. In fact, earlier reports mentioned the ability of acemannans to stimulate Th_2 cells. It has been postulated that the actions of acemannan may be attributed to the residual presence of aloerides (Pugh et al. 2001). In accord with this postulate, polysaccharides from crude extracts have been shown to enhance transcription of cytokines. High concentrations of aloeride also seemed to enhance macrophage activities (Pugh et al. 2001), and may be a contributing factor for the increased phagocyte stimulation by acemannan.

Traditionally, *Angelica sinensis,* because of its high phytoestrogenic content, is reputed to have a stabilizing effect on the female hormonal system, making it useful in treating menstrual problems. In China, it is often referred to as "female ginseng". Constituents of *A. sinensis* include ligustilide, butylidene phthalide, and β-sitosterol (Bensky and Gamble 1993). Essential oil extracts from *Angelica* were shown to inhibit selected pathogens (Elgayyar et al. 2001), and polysaccharides were shown to induce activation of both specific and nonspecific immune components (Ahn et al. 1998). In a later study, a polysaccharide, angelan, isolated from roots of *Angelica gigas*, was shown to trigger the release of cytokines IL-2, -4, -6, and INF-γ from macrophages. Cytokine release was found to occur in a sequential manner, with IL-6 presenting an almost immediate increase, followed by IL-4, with IL-2 having the slowest rate of increase (Sang et al. 1998). The increase in IL-2 may be attributed to the preceding increase in IL-6. In accord with the type of cytokines released, it can be postulated that with the initial rapid rise in mediators that activate Th_2 cells, the primary effect of angelan is the enhancement of T cell-dependent antibody production.

Ginger is a domestic remedy also known for its anti-infectant effects. Essential oil constituents from rhizomes of *Z. officinale* were found to decrease growth rate of a variety of bacteria and fungi, including *Staphylococcus* and *Candida* (Martins et al. 2001). The most effective antimicrobial constituent was found to be citral. Curcumene, a sesquiterpene, from ginger oil was found to inhibit *Rhizoctonia solani* (Agarwal et al. 2001). Another structurally characterized compound, 1,7-bis(4-hydroxy-3-methoxyphenyl)hept-4-en-3-one also showed inhibitory effects on

Pyricularia oryzae (Ramos et al. 1996). Ethanol soluble extracts from the rhizomes of *Z. officinale* were tested for their action on cytokines and found to promote the secretion of IL-1 and IL-6 in a time- and dose-dependant manner (Hori et al. 2003).

17.7 Monoclonal Antibody-Based Immunomodulator

Currently, there is only one antibody reagent licensed for use against an infectious disease in the United States — Palivizumab, although it is not used against fungal infections. Licensed in 1998, Palivizumab is a neutralizing, humanized monoclonal antibody (mAb) to protein F on respiratory syncytial virus (RSV). Because the antiviral activity of Palivizumab was associated with a reduction in inflammatory mediator release in a murine model of RSV (Mejias et al. 2004), its mechanism of action probably involves immunomodulation.

Recently, Mycograb, a human recombinant antibody fragment, was shown to significantly improve the response to amphotericin B in patients with invasive candidiasis (Pachl et al. 2006). Patients who received Mycograb and Amphotericin B showed a higher rate of complete overall response on day 10 of therapy, a significantly better mycological response and less *Candida*-attributable mortality than patients who received amphotericin B and a placebo. Mycograb is a recombinant antibody fragment lacking an Fc region, and is produced from a human anti-Hsp90 (heat-shock protein 90) cDNA library with an epitope that inhibits fungal Hsp90, NILKVIRKNIVKK (Matthews and Burnie 2001). Nonetheless, the *in vitro* activity of Mycograb (with amphotericin B and other antifungal agents) against resistant *Candida* and other fungal species (Matthews et al. 2003; Nooney et al. 2005) suggests it could hold promise as a broadly active antifungal agent.

The first mAb used to treat a fungal disease in humans was the mouse mAb 18B7, which binds to the cryptococcal capsular polysaccharide glucuronoxylomannan (Larsen et al. 2005; Casadevall et al. 1998). Extensive preclinical testing revealed that 18B7 augmented host defense mechanisms against *C. neoformans*, *in vitro* and *in vivo*, which has been reviewed by Casadevall et al. (2005). Although there is concern that mAbs could have limited usefulness for microbes that demonstrate high antigenic variation and mutability, combinations of mAbs have shown promise in overcoming this limitation (Ter et al. 2006).

17.8 Immune Peptides as Immunomodulators

Among the various categories of immunomodulating agents reported so far, certain peptides seem to hold better promise (Shukla et al. 1992). At least nine immune-defense peptide products are commercially available with annual sales of over $4 billion (Latham 1999). Six novel peptides viz., hexapeptide (89/215), glycopeptides (89/729, 90/341), pentapeptide (SP-5) and lipopeptides (86/450, 84/201), synthesized have been evaluated in CDRI for potent immunostimulant activity (Khan and Jain 2000). Hexapeptide 89/215, lipopeptide 86/450 and glycopeptide 90/341 provided marked protection to mice against systemic candidiasis. The peptides 86/450 and 84/201 also stimulated antibody and DTH in guinea pigs in the presence of Freund's complete adjuvant (Shukla et al. 1992). The lipopeptide (86/450) gave a sevenfold increase in HA titre, 135% increase in plaque-forming cells (PFC) and 218% increase in sheep red blood cells (SRBCs) in a mouse model. The 86/450 also induced nonspecific immunostimulation in the treated animals, as evidenced by the macrophage migration inhibition (MMI) and phagocytosis of (^{14}C) labeled *E. coli* of the peritoneal macrophage, and enhanced uptake of ^{3}H thymidine by the splenocytes of treated versus untreated normal mice (Djeu et al. 1986). Thus, 86/450 may have a direct stimulating effect on the lymphocytes.

17.9 Conclusion

As the world's immunodeficient population grows as a result of the HIV pandemic and increased use of highly immune suppressive regimens to treat a variety of illnesses, the challenges of mycotic infections are expected to continue. The increase in the population of compromised hosts, coupled with exciting biotechnology advances, has spurred on research into the immune response to fungi. The relative importance and interconnected responses of innate and adaptive immune in protection are actively being investigated. In order to develop a prospective chemotherapeutic agent against opportunistic infections, it is important to know that host factors such as degree of immunological debility as well as recovery of immune functions to normality may contribute significantly to a successful elimination of the pathogens. Concomitantly, methods of immune manipulation and reconstitution have become promising areas of research activity. Future therapies for invasive fungal may include agents that augment the antifungal activity of effector cells and alter Th balance. While there is some clinical experience with the use of recombinant cytokines as an adjunct to antifungal drug therapy (Roilides and Walsh 2004), clinical trials in highly compromised hosts are needed, as many questions remain regarding safety, efficacy, and optimal use. Another potential approach is manipulation of cellular signaling cascades.

During the last decade, immunomodulators have evolved to become a viable adjunct to established therapeutic modalities in infectious diseases. Immunomodulators of natural, synthetic, and recombinant origin can stimulate host defense mechanisms for prophylaxis and treatment of diverse viral, bacterial, parasitic, and fungal diseases. Many immunomodulators act by inducing endogenous production of cytokines. The therapeutic value of cytokines in infectious diseases is increasingly being recognized. Overall, the use of cytokines as therapeutic tools in the setting of infections has given rise to an optimistic view of the use of such reagents. Approaches based on neutralization of immunosuppressive cytokines in infectious diseases are also an area of considerable promise. Limitations of therapy with exogenous cytokines, however, have to be recognized. These are associated with the inherent toxicity of such material, their unclear pharmacological behavior, and their pleiotropic effects. Efficacy of exogenous cytokines capable of potentiating normal host defense mechanisms may be curtailed in immunocompromised patients lacking pertinent effector cells or having disease-related factors which prevent lymphocyte activation. In view of the short half-life of cytokines and high doses necessary to achieve therapeutic benefits, stimulation by chemically well-characterized immunomodulators of endogenous cytokines may be more advantageous. Selective stimulation by suitable immunomodulators of discrete lymphocyte subpopulations and cytokines important in protective effector mechanisms against a given infection will play an increasingly important role. Some immunomodulator preparations are already licensed for use in patients. Other compounds are being extensively investigated in preclinical and clinical studies. Nonantibiotic agents such as immunomodulators possessing antimicrobial activity offer a novel approach as an adjunct modality for the treatment of infectious and malignant conditions in the coming decades.

The future use of adjunctive immunomodulators for infectious diseases requires a better understanding of microbial pathogenesis and the relative need for immune activation versus immune modulation in the context of the immune response of the affected individual. In light of the fact that certain infectious diseases reflect an insufficient response, whereas others reflect an overly exuberant response, different types of interventions are likely to be required, depending on the immune status of the patient.

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