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Role of Fungal Virulence Factors in Evasion of Host Defenses

Contents

Abstract

Pathogenic fungi produce a number of virulence factors. A factor can be a "virulence factor" in the setting of a specific immunological defect but inconsequential to the infection in an immunocompetent host. This is an especially important concept considering that fungal infections are largely opportunistic in nature. There are a number of points in host defense that fungal virulence factors and secreted/shed

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fungal products can facilitate evasion: modulation of afferent phase cytokine signals (such as TNFα, IL-12, IFNγ, chemokines), altering antigen processing, blocking leukocyte recruitment to the site of infection, or inhibition of effector phase mechanisms. Fungal virulence factors and secreted/shed fungal products can interfere with a number of innate mechanisms, resulting in a dynamic interaction between microbe and host. Ultimately, this interplay between innate immunity, host genetics, and fungal virulence factors determines how the infection is initially handled and how adaptive immunity ultimately develops.

Key-words: immunity, cryptococcus, candida, paracoccidioides, leukocyte, lungs.

Fungal Virulence Factors

Pathogenic fungi produce a number of virulence factors (Table 1). A virulence factor can be defined as a microbial product that allows the microbe to evade being eliminated by the host. This definition differentiates "virulence factor" from a "factor required for virulence" such as growth at 37° C or a metabolic enzyme essential for growth. Furthermore, a factor can be a "virulence factor" in the setting of a specific immunological defect but inconsequential to the infection in an immunocompetent host. This is an especially important concept considering that fungal infections are largely opportunistic in nature. The topics of fungal virulence factors and microbial factors required for virulence are covered in depth in two excellent recent reviews (Hogan et al., 1996; Casadevall & Pirofski, 1999).

Table 1. (reviewed in Hogan et al., 1996; Casadevall & Pirofski, 1999; Ghannoum, 2000).

Fungal Virulence Factors
Adhesins
Proteases
Superoxide dismutase
Phospholipase
Pigments
Urease
Catalase
Toxins
Mannitol
Capsule
Cell Wall Components
Estrogen-binding Proteins

This review will concentrate on those fungal virulence factors that can modulate host immunity. There are a number of points in host defense that fungal virulence factors and secreted/shed fungal products can facilitate evasion:

A. Modulate afferent phase cytokine signals
    1. Down-regulate pro-inflammatory cytokines
    2. Up-regulate anti-inflammatory cytokines
    3. Promote switching of the immune response
B. Alter antigen processing
C. Block leukocyte recruitment to the site of infection
D. Inhibit effector phase mechanisms

These observations are supported by the well-documented observation that different strains of a fungal species can elicit very different types of immune responses, such as the differences in host immunity elicited by *Paracoccidioides brasiliensis* strain Pb265 and Pb18 (Vaz et al., 1994). In the larger context of microbiology, it should be kept in mind that pathogenic fungi are predominantly environmental microbes (with the possible exceptions of *Pneumocystis carinii* and *Candida albicans*). Thus, the primary role of fungal virulence factors is to enhance survival of the fungus outside the host (in the environment). However, the difference between pathogenic and non-pathogenic species of fungi that can grow at body temperature is the production of factors that enhance fungal survival in a host, i. e. virulence factors.

Innate Immunity to Fungi

Innate immunity is central to host defense against fungi even for fungal infections that require an adaptive immune response for clearance. Several broad effector mechanisms are induced early to combat the fungal infection and to promote subsequent signal generation. The cells of the innate immune system possess many immunoregulatory functions (providing a link between innate and adaptive immunity) along with potent anti-fungal effector mechanisms that can be activated by adaptive immunity.

The innate immune system employs broad, non-specific mechanisms to either completely destroy a microbial pathogen or contain the infection until an adaptive immune response is generated. Macrophages, neutrophils, and monocytes (i.e. phagocytic cells) are essential for resistance to fungal infections. NK cells, mast cells, and γδ T-cells are also important; however, their relative contribution is largely determined by the type of fungal insult. Non-hematopoietic cells such as epithelial

and endothelial cells also participate in innate immune surveillance against fungal infection. Many of the classical mechanisms of an innate response are utilized against fungi including: (1) the opsonization and phagocytosis by complement and pattern recognition receptors; (2) induction of oxygen-dependent and -independent fungicidal activity; and (3) production of pro-inflammatory mediators. The production of early cytokines by innate cells is a key aspect of the host response to fungal infection. These mediators play a critical role in activating phagocytes and orchestrating the development of adaptive immunity to fungi (Figure 1). Thus, innate immunity is an integral part of host defense against all fungal infections.

The cells of the innate immune system rapidly release cytokines in response to fungal products and binding of opsonized fungi. The phagocytic cells are important sources of TNFα, IL-12, MIP-1α, and IL-1 whereas NK cells and γδ T-cells can synthesize IFNγ. Epithelial cells and other structural cells are major sources of MCP-1. These early signals from innateimmunity:

a. result in cellular activation/maturation

b. result in cellular migration/recruitment

c. upregulate adhesion molecule expression

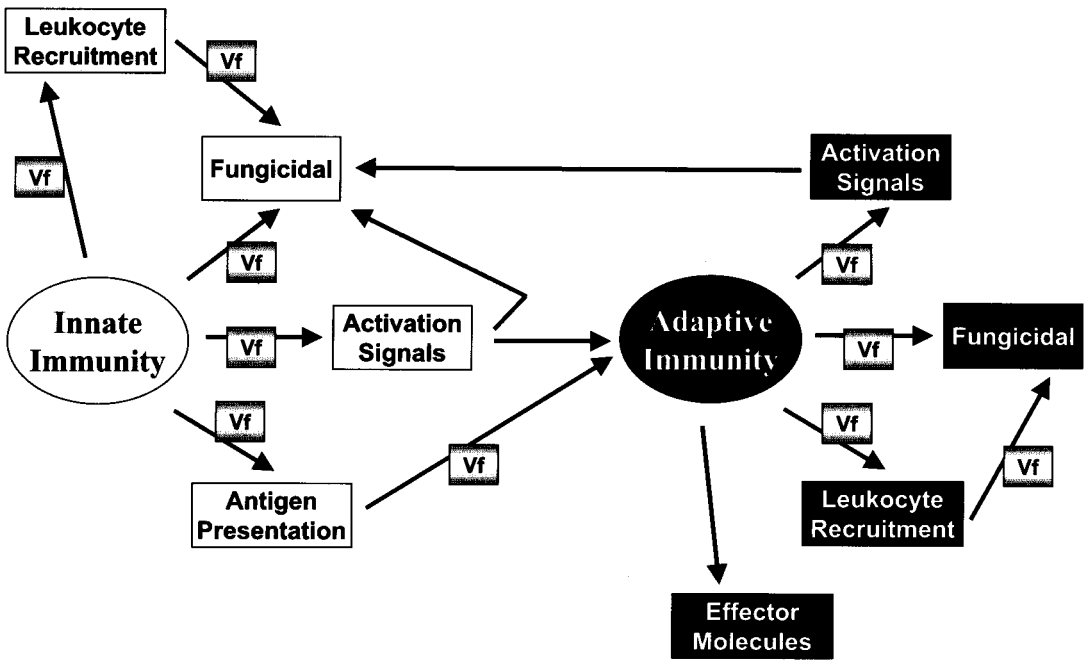

Figure 1. Anti-fungal host defense is the outcome of a network of signals from innate and adaptive immunity. A number of fungal virulence factors (Vf) have been identified which target particular pathways of host defense, thereby disrupting the network. This disruption can result in slowed clearance, chronic infection, or disseminating fatal infection.

Together, these signals focus the innate and adaptive response to the site of infection and enhance fungicidal activity of the recruited leukocytes; however, these signals are also the targets of many fungal virulence factors. (Figure 1).

Tumor Necrosis Factor α (TNFα)

TNFα is an early response cytokine, and several lines of evidence demonstrate a critical role for TNFα during fungal infections. The production of TNFα during pulmonary cryptococcosis is essential for resistance to *C neoformans*. TNFα is induced early in the course of a *C. neoformans* infection and preceedes the inflammatory response in infected mice (Huffnagle et al., 1996). Intraperitoneal administration of TNFα also increased the survival rate of animals infected with a highly virulent strain of *Cryptococcus* (Kawakami et al., 1996[a]). In contrast, neutralization of TNFα at the time of *C. neoformans* infection permanently skews the immune response and alters the production of subsequent innate signals. Administration of anti-TNFα monoclonal antibody at day 0 results in significant reduction of IL-12, IFNγ, and MCP-1 production at week 1 post-infection (Herring, unpublished observation). TNFα neutralization at day 0 also causes a shift from a Th1-type immune response to a Th2-like response in the lungs of infected mice. Anti-TNFα its treated mice fail to generate a DTH response to *C. neoformans* antigen and exhibit elevated IL-5 production and pulmonary eosinophilia (JL Lee et al., submitted).

A similar role for TNFα has been described in other fungal infection models. Serum TNFα levels of mice challenged with an avirulent strain of *P. brasiliensis* were significantly higher than those of mice challenged with a virulent strain and differences in the cell wall appeared to be responsible for the differences in TNFα induction (Figueiredo et al., 1993; Silva et al., 1994). Mice deficient in both TNFα and lymphotoxin develop a Th2 response to *C. albicans* infection and have high levels of IL-4 and IL-10 (Mencacci et al., 1998). The survival of these TNF/LT knockout animals was also significantly decreased and correlated with elevated organ CFUs (Netea et al., 1999). Mice deficient in the p55 TNF receptor were more susceptible to *P. brasiliensis* infection (Souto et al., 1999). In addition, neutralization of TNFα impairs resistance to *A. fumigatus* (Mehrad et al., 1999), *H. capsulatum* (Allendoerfer & Deepe, 1998), and *P. carinii* (Chen et al., 1992). TNFα can also be induced from cultured macrophages in the presence of *A. fumigatus* (Taramelli et al., 1996). Taken together, these findings strongly suggest that TNFα is a critical proximal mediator and is important for establishing host resistance to a variety of fungal pathogens.

Interleukin-12 and Interleukin-18

The importance of IL-12 in the early phase of fungal infections has been well characterized using *C. albicans*. In a murine model of systemic candidiasis,

induction of IL-12 RNA expression was detected in resistant mice but was undetectable in susceptible mice (Romani et al., 1994b). Furthermore, neutralization of IL-12 in the resistant animals resulted in a progressive, nonhealing infection and a shift to a Th2-type response (Romani et al., 1994a). These findings suggested that the early production of IL-12 plays a critical role in the development of protective Th1 immunity against *Candida*. Interestingly, neutrophils are an essential source of early IL-12 during an infection with *C. albicans*. Neutrophil depletion at the time of infection renders mice susceptible to *C. albicans* and induces a Th2 cytokine profile (Romani et al., 1997). Several studies have also described an essential role for IL-12 in establishing resistance to *C. neoformans* infection. *C. neoformans* can induce significant IL-12 production (Herring, unpublished observation) (Pitzurra et al., 2000). Administration of IL-12 during the first week of a cryptococcal infection increased long-term survival and prevented dissemination to the brain (Kawakami et al., 1996[c]). Conversely, anti-IL-12 treatment inhibited pulmonary clearance and caused a switch from Th1 to Th2 immunity (Hoag et al., 1997). IL-12 p35 or p40 knockout mice also exhibited T2 polarization in response to *Cryptococcus* (Decken et al., 1998). The induction of IL-12 appears to be important during *H. capsulatum* infection because IL-12 RNA is detected at day 3 post-infection (Cain & Deepe, 1998) and IL-12 neutralization increased the mortality to *Histoplasma* (Zhou et al., 1995). Thus, early production of IL-12 is critical for the initiation of protective Th1 immunity to fungi.

To date, the involvement of IL-18 in innate responses to fungi has not been extensively analyzed. The role of IL-18 in host resistance to *C. neoformans* was examined recently in IL-18 deficient mice. The levels of IL-12 and IFNγ were significantly reduced in the IL-18$^{-/-}$ animals and correlated with reduced clearance of *C. neoformans* from the lungs (Kawakami et al., 2000[a]). Similar results have been reported following neutralization of IL-18 during pulmonary cryptococcal infection (Kawakami et al., 2000[b]). In addition, administration of murine recombinant IL-18 enhanced fungal clearance from the lungs and prevented brain dissemination of a highly virulent strain of *Cryptococcus* (Kawakami et al., 1997[a]). These findings suggest that IL-18 plays a supporting role in host defense against *C. neoformans*. Based on this model of infection, it is likely that IL-18 will prove to be an important mediator in host responses to a variety of other fungal pathogens.

Interferon-γ (IFN-γ)

The production of IFNγ is essential for protection against fungi. IFNγ is a potent activator of fungicidal activity for cells of the innate immune system. However, IFNγ also drives Th1 responses to fungal infections. In experimental models of IFNγ

deficiency, diminished IFNγ levels result in a switch from protective Th1 to non-protective Th2 responses. For example, neutralization of IL-12 in a variety of fungal infection models results in significant reduction of IFNγ production (Cenci et al., 1998; Decken et al., 1998; Kawakami et al., 1996[c]; Zhou et al., 1995). TNFα neutralization also inhibits early IFN-γ production in the lungs of *C. neoformans* infected mice (Herring, unpublished observation). The importance of IFNγ in resistance to fungal infection has been strengthened further by studies performed in knockout animals. Mice deficient in IFNγ display increased organ burden and enhanced susceptibility to *C. albicans*, *H. capsulatum*, and *P. brasiliensis* (Allendoerfer & Deepe 1997; Balish et al., 1998; Kaposzta et al., 1998; Lavigne et al., 1998; Souto et al., 1999). A similar increase in mortality to *Cryptococcus*, *Aspergillus*, and *P. brasiliensis* has been demonstrated using anti-IFNγ Ab (Cano et al., 1998; Kawakami et al., 1996[b]; Nagai et al., 1995). IFNγ receptor knockout mice develop a Th2 bias and fail to generate protective Th1 immunity to *C. albicans* (Cenci et al., 1998). The presence of IFNγ also appears to be important for the induction of chemokines in response to *C. neoformans* (Kawakami et al., 1999). Therefore, the early production of TNFα and IL-12 induces IFNγ and a protective Th1 response is generated. Any perturbation of these early signals abrogates IFNγ production and shifts the response to a Th2 phenotype demonstrating that IFNγ is required for host resistance to fungi.

Role of Fungal Virulence Factors in Inhibition of Early Signal Molecules

Inhibition of these early signal molecules is a target for fungal virulence factors. Most of the work in the area of modulation of immunity by fungal virulence factors has concentrated on the factors produced by *C. neoformans*, but it is almost certain that many of the virulence mechanisms utilized by *C. neoformans* are also used by other fungi. The increased virulence of some strains of *C. neoformans* is largely due to inhibition of TNFα, IL-12, IL-18, and IFNγ production during the first week of infection (Huffnagle et al., 1995; Huffnagle et al., 1996; Kawakami et al., 1996[a]; Kawakami et al., 1997[b]). As mentioned previously, strain virulence differences in *P. brasiliensis* correlate well with the ability to block TNFα induction (Figueiredo et al., 1993). Intraperitoneal administration of TNFα, IL-12, or IL-18 can increase survival and decrease dissemination of these cryptococcal strains (Kawakami et al., 1996[a]; Kawakami et al., 1997[a]; Kawakami et al., 1996[c]). Addition of polysaccharide capsule from *C. neoformans* decreases TNFα and IL-1b production by macrophages *in vitro* (Vecchiarelli et al., 1995). Capsule also induces IL-10 production thereby providing a mechanism by which capsule may down-regulate macrophage activation and protective T1 responses (Levitz et al., 1996; Vecchiarelli et al., 1996; Monari et al.,

1997). Melanin can also reduce TNFα production by alveolar macrophages (Huffnagle et al., 1995). NK cell production of TNFα is also inhibited by co-culture with cryptococci (Murphy et al., 1997). The polysaccharide capsule and melanin also interfere with effector phase killing mechanisms such as phagocytosis and oxidant-mediated killing (Kozel & Mastroianni 1976; Jacobson & Tinnell 1993; Wang & Casadevall 1994).

Chemokines and Leukocyte Recruitment

The production of chemotactic factors at the site of a fungal infection is critical for effective recruitment of leukocytes to that site. A number of molecules are chemotactic for leukocytes and these molecules can be categorized into two groups. The first group includes (a) peptides derived from activation of the complement pathway (C3a, C5a, and C5a-desArg), (b) leukotrienes, and (c) fungal products. The second group includes chemokines, a supergene family of small inducible peptides with potent chemotactic activity for leukocyte subpopulations (reviewed in O'Garra et al., 1998; Murphy et al., 2000; Rossi & Zlotnik, 2000; Zlotnik & Yoshie, 2000). Chemokines can be induced either directly or indirectly by fungi or fungal products and there is a ever-growing body of data supporting the important role of chemokines in leukocyte recruitment and development of immunity during fungal infection (reviewed in (Traynor & Huffnagle, 2000).

Fungal virulence factors can also interfere with leukocyte recruitment and antigen presentation. Cryptococcal polysaccharide and melanin both interfere with antigen presentation and subsequent cytokine signaling by macrophages for *Cryptococcus*-specific lymphocyte proliferation (Collins & Bancroft, 1991; Vecchiarelli et al., 1994; Huffnagle et al., 1995). In addition, cryptococcal antigen and polysaccharide induce immunologic unresponsiveness to subsequent challenge with *C. neoformans* (Murphy, 1989). Another mechanism of evasion is by blocking adhesion molecule-leukocyte interactions on the endothelium, thereby interfering with leukocyte recruitment to the site of infection (Dong & Murphy, 1996). Chemokine production by endothelial cells is also inhibited by live, but not killed, *cryptococci* (Mozaffarian et al., 2000). Thus, pathogenic fungi possess mechanisms to modulate leukocyte recruitment to the site of infection and the future study of these interactions should yield significant insight into the mechanisms of immune evasion by fungi (resulting in chronic infection).

Other Fungal Virulence Factors

Other factors important in fungal virulence include yeast to hyphal transformation in *C. albicans* and genes such as *STE12*a in *C. neoformans.* The germinated hyphal form of *C. albicans* is better able to adhere to and penetrate host tissues than the yeast form (Sandin, et al., 1982; Sobel et al., 1984). *STE12*a controls

the expression of several phenotypes known to be involved in virulence, such as capsule and melanin production, but the role of *STE12*a in virulence may also depend on the relative expression of non-*STE12*a controlled genes (Yue et al., 1999; Chang et al., 2000). Phospholipase B activity has been proposed to play a role in virulence of *C. albicans* and *C. neoformans* (Ghannoum, 2000). In addition, estrogen-binding proteins have been identified in a number of fungi including *P. brasiliensis*, *C. albicans*, and *C. imitis* (reviewed in (Hogan et al., 1996). Our laboratory has also recently identified the production of bioactive lipids by pathogenic yeast and other fungi (Noverr et al., submitted). Thus, fungal virulence factors and secreted/shed fungal products can interfere with a number of innate mechanisms, resulting in a dynamic interaction between microbe and host. Another variable in the outcome of an infection is the genetics of the host. However, the role of virulence factors in genetic susceptibility remains a relatively unexplored venue of research. Ultimately, this interplay between innate immunity, host genetics, and fungal virulence factors determines how the infection is initially handled and how adaptive immunity ultimately develops (Figure 2).

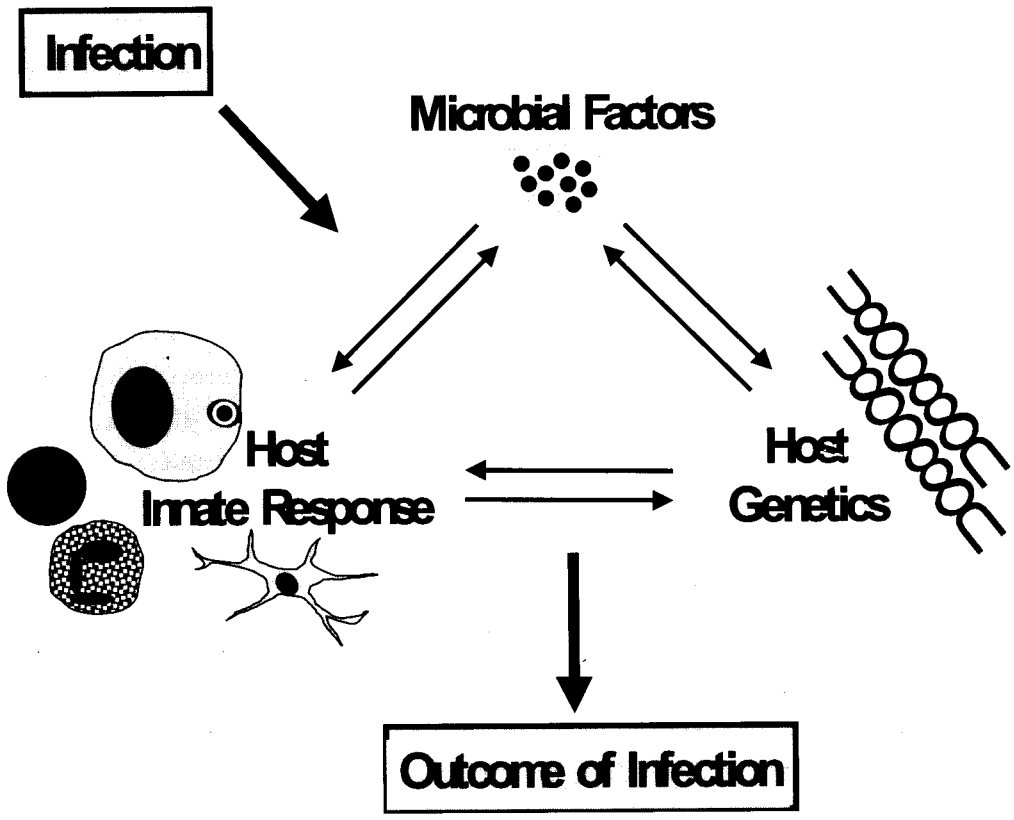

Figure 2. The interplay between innate immunity, host genetics, and fungal virulence factors determines how the infection is initially handled and how adaptive immunity ultimately develops. Each of the three components of this triad has the potential to influence the other components or to be influenced by the other components. Thus, the outcome of a fungal infection is determined by this dynamic response.

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