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Overviews

Mammalian Model Hosts of Cryptococcal Infection

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The rising incidence of serious fungal diseases represents a growing threat to human health. *Cryptococcus neoformans*, an encapsulated yeast saprophyte with global distribution, has been recognized as an important emerging pathogen. Humans frequently develop asymptomatic or mild infection with *C. neoformans*, but individuals with impaired host defense systems may develop severe pneumonia and potentially fatal meningoencephalitis. Insight into the biology and virulence of *C. neoformans* is advancing rapidly and will be propelled even further by the recently completed and published genome sequences for two related strains of *C. neoformans* serotype D. Several mammalian model hosts including the guinea pig, rabbit, rat, and mouse have been developed for the study of cryptococcosis. The combination of microbial genomics with well-characterized model hosts that are amenable to immunologic and genetic manipulation represents a powerful resource for comprehensive study of cryptococcal disease pathogenesis as well as vaccine and antifungal drug therapy. This review provides an introduction to each mammalian model host and briefly highlights the advantages, limitations, and potential of each system for future research involving cryptococci.

Abbreviations: CFU, colony-forming units; CSF, cerebrospinal fluid; Th, T helper cell

Fungi are eukaryotic microorganisms found ubiquitously in the environment as single-celled yeasts or as the multicellular filamentous structures known as molds. Among the many thousands of fungal species that have been identified, fewer than 20 are considered pathogenic for humans. Fungal diseases, or mycoses, are of particular concern for people who have impaired immunity. Indeed, mounting evidence indicates that the incidence of invasive fungal infections has been increasing during the past 3 decades, largely as a result of an aging population coupled with advances of medical therapy that together have dramatically increased the burden of chronic comorbid disease.[27] The growing medical relevance of these microorganisms is compounded by the fact that relatively few options exist for the therapy of human mycoses. Together, these factors have facilitated the emergence and re-emergence of serious disease caused by agents such as *Coccidoides immitis*, *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans*.[32]

C. neoformans is a yeast pathogen within the phylum Basidiomycota that was first identified as a cause of human disease over a century ago and has since been identified as a pathogen throughout the world. Increasing scientific interest in this microorganism is reflected by several comprehensive reviews of *Cryptococcus* and cryptococcosis that have recently been published.[57,74,98] The taxonomy and classification of *Cryptococcus* has undergone considerable evolution during the past half-century. After the initial discovery of distinct mating types that were capable of producing fertile spores under specific conditions, *C. neoformans* was separated into 2 varieties with distinct capsular serotypes, var. *neoformans* (serotypes A and D) and var. *gattii* (serotypes B and C). Proponents of phylogenetic studies using DNA sequence variation subsequently proposed as many as 8 major molecular types of *Cryptococcus*.[34,87,88] The most appropriate classification remains controversial; however, on the basis of biologic, morphologic, and phenotypic criteria, it has been suggested that a 2-species concept incorporating the capsular serotype represents the most straightforward system. In this proposal, the genus *Cryptococcus* is composed of *C. neoformans* serotypes A, D, and AD along with *Cryptococcus gattii* serotype B and C.[65]

C. neoformans is a free-living organism that has been recovered from tropical and temperate climate zones throughout the world. Soil contaminated with avian excreta frequently is reported as a primary ecologic site for *C. neoformans*, although it is unclear whether the organism originates from bird guano. Interestingly, the high density of pigeons in urban areas and an elevated rate of childhood seroconversion among city dwellers also suggest a link between exposure to bird guano and infection with *C. neoformans*.[44] Other ecologic sources that have been associated with this saprophyte include decaying wood and plant material.[108] Phagocytic amoebae in the soil may ingest free-living *C. neoformans* and provide a natural selection mechanism for yeasts that are able to survive an intracellular environment, thereby maintaining their virulence for humans.[117]

Prior to the recognition of immunosuppression caused by human immunodeficiency virus, cryptococcosis was a relatively rare disease. For example, for the period 1971 to 1980, an incidence of less than 1 case per million persons per year was documented in Northern California.[37] After the onset of the human immunodeficiency virus epidemic in 1981, cryptococcosis quickly became a defining illness of acquired immunodeficiency syndrome, with rates as high as 13.3% among infected people.[100] In North America, the introduction of highly effective antiretroviral therapy in the early 1990s led to an overall decline in the prevalence of opportunistic infections including cryptococcosis.[100] Nevertheless, in geographic areas with little access to antiviral therapy, *Crypto-*

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coccus remains a leading cause of community-acquired meningitis within the population infected with human immunodeficiency virus.[48] Other predisposing factors for cryptococcal infection include solid-organ transplantation,[56] anti-inflammatory treatment,[47] and hyperimmunoglobulin M immunodeficiency.[58,120] In addition, cases of pulmonary and extrapulmonary cryptococcosis are now being reported more frequently among apparently immune-competent individuals.[79,91]

Virulence Factors

Outside the host, *C. neoformans* is believed to exist as a poorly or moderately encapsulated spherical to oval structure with a diameter ranging from 2 to 10 µm. Human infection is believed to occur via inhalation and colonization of the distal alveolar spaces of the lung by infectious propagules consisting of cells or basidiospores.[57,118] The pathogenesis of *C. neoformans* is mediated by 4 main virulence factors that allow the yeast to survive within the host environment; these include the ability to grow at 37 °C,[64] synthesis of an extracellular capsule,[21] production of melanin,[125] and secretion of degradative enzymes.[24,28,29,35,57] The innate ability of *C. neoformans* to grow at 37 °C is an essential, calcineurin-dependent characteristic that allows it to proliferate and cause disease.[96] In addition, the expression of a unique polysaccharide capsule, the most distinctive and intensively studied virulence factor possessed by *C. neoformans*, is enhanced at this temperature.[126] The major capsular structure consists of an unbranched chain of mannose residues substituted with xylosyl and β-glucuronyl groups that allows *C. neoformans* to resist opsonization and phagocytosis; shedding of capsule during infection also may subvert the immune response by altering the host chemokine and cytokine secretion profile.[57] *C. neoformans* shares the ability with other pathogenic fungi to produce melanin from diphenolic compounds by use of the enzyme laccase. Melanin may allow the fungus to resist host oxidative stress, particularly generated by phagocytic cells. Progressive infection by *C. neoformans* is characterized by hematogenous dissemination from its site of entry in the lungs to various organs including the brain, skin, kidney, and liver.[19] Studies that examine host immunity generally use either a high-virulence encapsulated strain such as *C. neoformans* H99 (serotype A) or a moderately virulent encapsulated strain such as *C. neoformans* 52D (serotype D), both of which are commercially available (American Type Culture Collection, Manassas, VA; www.atcc.org). Because of their low disease-causing potential, unencapsulated strains are generally not used for studies in immune-competent model hosts.

Pathology and Immunology

The ubiquitous environmental presence of *C. neoformans*, combined with serologic evidence of extensive host exposure yet a low incidence of clinical disease, suggests that a competent host immune response is usually successful in clearing cryptococcal infection.[19] Highly phagocytic alveolar macrophages present within the air spaces of the lung represent a frontline of host defense against inhaled cryptococci.[115] Activation of macrophages by cryptococcal infection rapidly elicits chemokine-mediated recruitment of neutrophils and monocytes to the lungs and into the airways in an effort to contain and prevent dissemination. Protective immunity requires a T helper cell (Th) 1 pattern of cytokine- and lymphocyte-mediated adaptive immune response.[54] A variety of cytokines including tumor necrosis factor α, interleukin 12, interleukin 18, granulocyte–macrophage colony stimulating factor, interferon γ, macrophage inflammatory protein 1, and monocyte chemoattractant protein 1 have all been implicated in the development of effective host defense,[1,30,33,53,60,70] whereas clearance of circulating antigen as well as opsonization of cryptococci are mediated by humoral factors including antibody and complement.[83,123,124] Despite antifungal treatment, immunosuppressed patients that survive an initial infection may develop a chronic or relapsing form of meningoencephalitis.[8] Although studies of the human immune response have been highly instructive, limitations inherent to this approach have restrained progress toward a complete understanding of cryptococcal disease pathogenesis. Accordingly, model hosts represent an important tool that will enable comprehensive investigation of human cryptococcal disease as well as its treatment.

Animal Models

General considerations. Apart from its importance as a cause of human infectious disease, *C. neoformans* is also a wide-ranging veterinary pathogen that naturally infects several common mammals, including cats, dogs, cows, horses, and primates, as well as a variety of invertebrate species. The occurrence of both sporadic and epidemic animal infections with clinical manifestations that resemble human illness undoubtedly has contributed to the development of several excellent and diverse model systems for the study of cryptococcosis. Each animal host confers specific advantages, as well as limitations, for modeling of human disease; therefore several considerations may apply when selecting a specific model (Table 1). For example, choosing a model host that is naturally susceptible to infection in its usual environment may be relevant for vaccine efficacy studies. Use of a naturally resistant animal species such as the rabbit for this purpose would not be feasible without potentially undesirable experimental manipulations such as immune suppression. In addition, the choice of model host may influence the type and severity of clinical manifestations that can be analyzed in response to a standard cryptococcal challenge. Larger animals such as the rat and rabbit are suitable for studies of meningoencephalitis because their brains are easily accessible to experimental manipulation. In comparison, mice are a preferred system for studies of pneumonia because of the availability of comprehensive genetic and immunologic reagents. Finally, practical limitations such as cost and complexity of animal maintenance may be a primary consideration with large species, such as primates.

Regardless of the model host that is chosen, its role in the study of complex host–pathogen interactions may vary according to the specific research objectives. For example, a so-called 'host-centric' approach focuses primarily on the contribution of inflammatory and immune responses by the animal species in disease pathogenesis after a standardized microbial challenge.[17] Conversely, a 'microbe-centric' approach typically is used to investigate disease-causing factors associated with the pathogen in the setting of a uniform model host system.[45] Such divisions are likely to diminish in the future as the existing diversity of animal models unites with the potential for genetically modified cryptococci, resulting in truly comprehensive host–pathogen interaction studies. In addition, comparative analysis of diverse model systems likely will provide valuable insight into the evolution of host defenses and clarify the hierarchy of immune responses that are necessary for definitive protection from fungal infection.

Both mammalian and nonmammalian model hosts are ame-

Table 1. Characteristics of mammalian models of *C. neoformans* infection

	Guinea Pig	Rabbit	Rat	Mouse
Weight	350–450 g	2–3 kg	250–350 g	20–40 g
Routes of infection	Intravenous[95]	Retinal via carotid artery[38]	Intraperitoneal[41]	Intraperitoneal[110]
	Intraperitoneal[112]	Intracisternal[104]	Intracisternal[41]	Intravenous[110]
	Intratracheal[15]	Intratracheal[92]	Intratracheal[46]	Intranasal[2]
		Intratesticular[6]		Intracerebral[9]
				Intratracheal[51]
Immunosuppression	Not required	Corticosteroids required[99]	Not required	Not required
Phenotypes of alveolar macrophages	Phagocytosis is inhibited by cryptococcal capsular polysaccharide[15]	Evidence of oxidative metabolism[92]	Phagocytosis causes an increase in oxidative burst and enhanced restriction of intracellular growth in comparison to mouse[115]	Macrophage depletion ameliorates fungal burden[115]
	Unable to kill ingested yeast[15]	Low phagolysomal pH upon phagocytosis[92]	Macrophage depletion enhances fungal burden[115]	
Advantages	Docile nature[12]	Requirement for immune suppression similar to humans	Large size allows non-surgical intratracheal infection[42]	Extensive immunologic and genetic resources available[11]
		Large body size allows repeated body fluid sampling and drug administration[99]	More resistant to pulmonary infection than mice[115]	Well-characterized inbred strain response to infection[51]
			Availability of inbred strains[84]	Relatively inexpensive to purchase
Disadvantages	Limited number of inbred strains	Expensive to purchase and maintain[12]	More expensive than mice	Small body size may hinder procedures
	Limited immunologic agents	Requires a large infectious dose and immune suppression[104]	Fewer immunologic agents than mouse[42]	
		Limited immunologic and genetic information available[99]		

nable to the study of *C. neoformans* virulence and disease pathogenesis. The various nonmammalian model hosts for study of cryptococcosis have the potential to yield unique and key insights into ancient or highly conserved mechanisms of host defense.[76] Conversely, the marked evolutionary divergence of these systems from those of mammals may impose limitations on the accurate modeling of disease in humans. From this perspective, mammalian model hosts have distinct advantages owing to their extensive anatomic, physiologic, and immunologic similarities with humans.

Among the routes that have been used for experimental cryptococcal infection in mammals, the most convenient yet least clinically relevant method is intraperitoneal injection, despite the observation that it has potential to induce disseminated disease.[89] Meningoencephalitis, the most important manifestation of human cryptococcal disease, has been most extensively studied by use of direct intracerebral or intracisternal injection.[9,43,104] Pneumonia has been modeled through intranasal or direct intratracheal inoculation.[89] Intravenous infection is perhaps the most quantitative method for infection and mimics hematogenously disseminated disease. Individual model host characteristics, including the ability to deliver a reproducible infectious dose as well as the likelihood of establishing a clinical disease, are relevant factors in the choice of a specific route for experimental infection.[89]

Biocontainment and housing needs. To prevent inadvertent contamination with or accidental occupational exposure to potentially dangerous microbes, appropriate precautions are required for experimental models that involve an infectious agent. *Cryptococcus* sp. is classified as Biosafety Level 2 pathogen and therefore is considered a moderate hazard to personnel and the environment.[111] One study using a mouse model of cryptococcal infection demonstrated contamination of bedding when C. neoformans was administered by an intratracheal route.[94] The precise mechanism for fungal spread was not identified but may have been due to sneezing or grooming behavior. Transmission to sentinel animals housed with infected mice was not demonstrable despite the use of a highly sensitive assay, raising questions about the significance of this observation. Despite such uncertainty, personnel should be prudent when cleaning cages and disposing the bedding of infected animals, and immunocompromised people should avoid animal rooms housing mice with pulmonary *C. neoformans* infection. Decades of experience from numerous laboratories around the world indicate that the actual risk of personnel becoming infected with *C. neoformans* is relatively small; however, current recommendations include the use of a class II biosafety cabinet for the manipulation of *C. neoformans*, along with filter-top cages for infected animals.[111] Published cases of accidental infection among laboratory workers thus far have been limited to cutaneous injury with heavily contaminated syringes.[18] Accidental needle stick exposures should be immediately managed with

prophylactic antifungal medication to treat the local site of injury and prevent the dissemination of any deposited yeast.[18]

Outbred versus inbred mammalian models. Inbred strains (genetically homozygous animals) and outbred stocks (closed populations that are bred to maintain maximal heterozygosity) are both used to study infectious diseases.[5,14,25] The selection and genetic makeup of a model host may influence experimental findings. Outbred animals have been used extensively in the fields of pharmacology and toxicology; the inherent genetic heterogeneity of these animals contributes to the overall fitness of the model host and is reflective of natural populations. Conversely, outbred stocks may be disadvantageous when attempting to replicate an experiment that requires a stable host genetic background, because phenotypic variation will be subject to the influence of heritable as well as environmental factors. The limited genetic drift within an inbred strain also facilitates the reliability and reproducibility of experimental results in long-term experiments. Furthermore, the contribution of host genetic factors within a model can be investigated through comparisons of several inbred strains that exhibit differing phenotypic traits.[16]

Antifungal drug evaluation. Human cryptococcal meningitis remains a common opportunistic infection among immunosuppressed persons, with a mortality of 10% to 30% despite antifungal drug treatment.[7] The current recommendation for initial therapy of severe meningitis in humans is amphotericin B, a polyene fungicidal agent, in combination with flucytosine for 2 wk, followed by consolidation therapy with fluconazole. Animal models have been instrumental in determining the potential for drug efficacy in human cryptococcal disease.

Cortisone-treated New Zealand White rabbits have been used most commonly to model chronic cryptococcal meningitis. In this system, viable cryptococci are injected directly into the cisterna magna by use of a fine-gauge needle, and cerebrospinal fluid (CSF) is sampled at various intervals to determine quantitative fungal growth and parameters of inflammation.[104] Despite the obligatory requirement for immunosuppression, untreated rabbits develop fatal basilar meningitis with mononuclear pleocytosis that resembles human disease, and rabbits are large enough to permit serial CSF examinations for evaluation of drug efficacy.[59,102,103,105] Differential effects of immunosuppression have also been described by using rabbits: corticosteroid administration led to a striking reduction in CSF and peripheral blood leukocytes, whereas cyclosporine induced a functional interleukin 2-dependent defect without leukopenia.[101] In this study, rabbits treated with cyclosporine progressed to death more rapidly than did those treated with cortisone.

Mice also have been used extensively as models for evaluation of clinically relevant drugs, including combination treatments such as amphotericin B or fluconazole with flucytosine,[49,66,67,93,107] and the development of a therapeutic monoclonal antibody directed against the cryptococcal capsular polysaccharide.[68] In addition, 1 study used Dunkin-Hartley guinea pigs to evaluate the efficacy of an intravenous formulation of itraconazole, a second-line agent for human cryptococcosis.[95]

In the subsequent sections, we briefly describe 4 common mammalian model hosts for experimental cryptococcal infection. This information likely will be pertinent to the assessment and effective use of each model host in future studies of cryptococcal disease pathogenesis.

Guinea pig (*Cavia porcellus*). Guinea pigs are amenable to infectious modeling because of their relatively tame nature, medium body size, and susceptibility to a variety of microorganisms.[12] The guinea pig was the first animal model system selected for the study of cryptococcosis, and guinea pigs also have been used to model various other invasive fungal infections, including zygomycosis,[122] aspergillosis,[4] and candidiasis[80] as well as mycobacterial disease.[4]

Intranasal challenge of female guinea pigs with a low dose (10^3 colony forming units [CFU]) of a clinical isolate of *C. neoformans* in the presence or absence of epithelial disruption was insufficient to induce disease or elicit a demonstrable immune response.[73] Another study showed that adult Dunkin-Hartley guinea pigs do not exhibit noteworthy clearance of nonencapsulated *C. neoformans* from the lungs 6 h after intratracheal administration of 1.5 to 1.7×10^7 CFU.[15] This in vivo phenotype was explained by the observation that in vitro phagocytosis of nonencapsulated cryptococci by quiescent and activated alveolar macrophages was associated with a failure to kill the ingested organism.[15] Intraperitoneal infection with a high dose (10^7 CFU) of *C. neoformans* 101/78A (from the stock culture collection of the Universidad Nacional de Córdoba, Cordoba Argentina) resulted in consistent dissemination to the lungs and brain with variable dissemination to the spleen, liver, and kidney at 40 d after infection in guinea pigs of both genders.[112] Mononuclear cell infiltration was noted in all tissues that were culture-positive for *C. neoformans*.[112] With the guinea pig model, female guinea pigs were more resistant to *C. neoformans* than males following intraperitoneal infection, a finding that is consistent with a similar gender effect in human cryptococcal disease susceptibility.[31] Disseminated cryptococcal disease induced by the intravenous route of infection also has been used in outbred Dunkin-Hartley guinea pigs to test the efficacy of antifungal therapy.[95] In that study, control animals infected with *C. neoformans* B42419 at a dose of 200 CFU/g of body weight developed infection of the brain, lymph nodes, muscle, and skin.[95] Administration of corticosteroids to infected guinea pigs has revealed the important role of cell-mediated immunity in host resistance against *C. neoformans*,[31] a finding that is consistent with studies in rabbits,[104] rats,[39] and mice,[77] but a detailed analysis of the guinea pig immune response has not been performed.[112]

The genome of the guinea pig has been sequenced and assembled as part of the Mammalian Genome Project by the Broad Institute.[13] This resource undoubtedly will be useful for comparative in silico studies of candidate cryptococcal susceptibility genes identified in other models. Because only a few inbred guinea pig strains exist (strains 2 and 13 are used most often), evaluation of the biologic relevance of these putative host factors to the guinea pig immune response against *Cryptococcus* may be far more limited.[36] Another disadvantage of the guinea pig model is the paucity of immunologic reagents and genetic tools that are available for analysis of disease pathogenesis.

Rabbit (*Oryctolagus cuniculus*). The rabbit has been used as a model host for several fungi including *Aspergillus*,[97] *Candida*,[106] and *Cryptococcus*.[99,104] Cryptococcal endophthalmitis, a rare clinical condition, was modeled in healthy outbred New Zealand White female rabbits by administrating 10^7 to 10^8 CFU of *C. neoformans* 4877 serotype D directly into the carotid artery.[38] The large dose and direct route of infection were unique aspects of this model that resulted in pathology of the iris, vitreous humor, and optic nerve radiations.[38] An important characteristic of rabbits is their natural resistance to cryptococcosis; this feature is partially attributable to their relatively high normal body temperature (39.3 to 39.5 °C), which inhibits fungal replication and

Table 2. Genetic loci that regulate host resistance to mouse *C. neoformans* infection

Locus symbol	Mutant allele	Chromosomal location	Function or phenotype	Reference(s)
Hc	Hc^0	2	Hc^0 encodes a defective fifth component of complement (C5)	109, 110
IgH complex-linked	Undefined	12	Unknown function; CB.17 is a BALB/c strain with a congenic segment from C57BL/6	78
Btk	Btk^{xid}	X	Bruton's tyrosine kinase; *xid* allele impairs B lymphocyte development and function	82
Lyst	$Lyst^{bg}$	13	Lysosomal trafficking regulator; resembles human Chediak-Higashi syndrome	81
FoxN1	$FoxN1^{nu}$	11	Encodes the forkhead box N1 transcription factor	20, 114
Prkdc	$Prkdc^{scid}$	16	Deficient B and T lymphocyte function	10
H2	$H2^k$	17	Major histocompatibility complex haplotype	85

dissemination via the respiratory tract. The intratesticular route of administration has been used to target an area of lower body temperature,[6] but this approach does not reflect a natural route or site of disease. Immune suppression with corticosteroids is now commonly used to circumvent intrinsic host resistance and more effectively model progressive cryptococcal disease in rabbits. Although the need for corticosteroids may be a disadvantage, most infected humans also have some form of impaired immunity.

The large size of rabbits allows for frequent, repetitive sampling of CSF, facilitating the investigation of chronic cryptococcal meningitis. In New Zealand White male rabbits treated with cortisone and then inoculated with *C. neoformans* directly into the subarachnoid space of the animals, survival was inversely related to the dose of cortisone received, and noteworthy morbidity or mortality did not occur in untreated animals for 6 wk after infection.[49,104] Clinically relevant pathologies included basilar meningitis, cellular infiltration into the CSF that was inversely related to cortisone dose, and a mild fever.[104] The utility of the rabbit model of infection via subarachnoid injection combined with the docile nature and large size of the rabbit has allowed researchers to investigate the efficacy of various antifungal medications for the treatment of cryptococcal meningitis.[102,104] In addition, local pulmonary immunity has been investigated by examining the interactions of rabbit alveolar macrophages with *C. neoformans* 24 h after intratracheal infection.[92] Cells obtained by lung lavage of male rabbits exhibited marked phagocytosis, increased oxidative metabolism, and lower phagolysosomal pH that together are suggestive of an activated anticryptococcal phenotype.[92]

Potential limitations of the rabbit model host include its high maintenance cost, the availability of only a few inbred strains, and the requirement for immune suppression. Conversely, sequencing and assembly of the rabbit genome likely will facilitate comparative genomic and immunologic studies with other mammals. Rabbits will continue to play a valuable role in therapeutic drug studies because of the strong correlation between their response to antifungal treatment and human disease outcomes.[99]

Mouse (*Mus musculus*). Inbred mice are the most popular model host for laboratory investigation of cryptococcal infection.[75] Prominent advantages of this model host include its relatively low maintenance cost and ease of handling, the availability of numerous inbred strains with well-developed tools for immunologic and genetic analysis, and the ability to produce a clinically relevant experimental cryptococcal infection. Inbred strains of mice are highly susceptible to cryptococci through the intratracheal, intranasal, intravenous, and intraperitoneal routes of infection without requirement for immune suppression.[2,51,110] A number of naturally occurring genetic defects involving the immune system have been associated with extreme susceptibility to progressive cryptococcosis (Table 2). Comparative analysis of commonly used immunocompetent inbred mice has revealed dramatic interstrain variation in the susceptibility to progressive cryptococcal disease; this characteristic is advantageous for studies of genetically regulated host defense factors.[78,109,110] Resistance to progressive *C. neoformans* lung infection in the mouse is associated with the development of a Th1 pattern of adaptive immunity characterized by expression of tumor necrosis factor α,[1] interleukin 12,[30] interleukin 18,[60] granulocyte–macrophage colony-stimulating factor,[70] macrophage inflammatory protein 1α,[33] and monocyte chemotactic protein 1.[53] Conversely, the susceptible inbred strain C57BL/6 develops an interleukin 5-dependent pulmonary eosinophilia as early as 1 wk after intratracheal infection with *C. neoformans*.[51] Similar to humans with cryptococcosis, susceptible mouse strains develop disseminated disease after experimental lung infection.[8,55] In addition, both intravenous and intracerebral routes of infection have been used in the mouse to model *C. neoformans* dissemination. Use of the intravenous technique confirmed that a weakly encapsulated form of virulent *C. neoformans* crosses the blood–brain barrier and causes meningoencephalitis shortly after entering the blood stream.[22,26] Studies using a direct intracerebral route of infection have demonstrated an important role for phagocytosis of yeast within the central nervous system in mouse survival after cryptococcosis.[10]

In vitro investigations have demonstrated that mouse neutrophils and alveolar macrophages have the ability to phagocytose and kill *C. neoformans*.[23,71] Somewhat surprisingly, a recent study comparing 3 inbred strains of mice with 3 rat strains demonstrated that depletion of alveolar macrophages 3 d before infection reduced fungal burden in the mouse lung but increased it in the rat.[115] These data suggest that alveolar macrophages execute species-specific roles within the model host that may either promote chronic infection or facilitate the clearance of *C. neoformans.*

The extraordinary power of mouse immunologic and genetic tools has advanced the understanding of the role of host defenses during cryptococcal infection. Despite the known differences between mouse and human immunology, the contribution of individual cytokines, chemokines, and cell populations to effective anticryptococcal immunity has been studied extensively by use of antagonistic antibodies and various genetically engineered knockout strains.[86] The overall conclusion arising from these studies is that robust cell-mediated immunity interacting with humoral host defenses is crucial for protection and clearance of cryptococcal infection.[52,63,90,123]

Rat (*Rattus norvegicus*). The rat is a well-established model host for the study of chronic or latent pulmonary cryptococcal infection with the potential for reactivation after corticosteroid administration.[42,45,46,89] Direct intracisternal inoculation also has been used to model cryptococcal meningitis in the rat.[43] Intratracheal *C. neoformans* infection of the rat reproduces many of the histopathologic and serologic features of human cryptococcal pneumonia, including cellular recruitment, granuloma formation, minimal extrapulmonary dissemination, and low levels of capsular polysaccharide in the serum.[41] Macrophage-derived monocyte chemoattractant protein 1[50] and inducible nitric oxide synthase[40] mediate cellular recruitment to the lung after cryptococcal infection. The initial host response is sufficient to contain the infection, but intracellular persistence and long-term (longer than 18 mo) survival of cryptococci occur and are associated with downregulation of cellular and humoral immunity.[45] In contrast to most inbred mouse strains, immunocompetent rats do not predictably disseminate a pulmonary infection after intratracheal administration of *C. neoformans*, indicating that rats may be inherently more resistant to progressive cryptococcal disease.[41,42] Comparative studies indicate that these host susceptibility differences may be partially attributable to the enhanced anticryptococcal activity of rat alveolar macrophages relative to those of mice.[115]

A dual role of transforming growth factor β1 on cryptococcal clearance has recently been described through use of the rat model.[116] This pleiotropic cytokine plays a role in several chronic infectious diseases and has macrophage deactivating properties that can promote persistent infection.[72] Administration of transforming growth factor β1 promotes cryptococcal growth when given at the time of the infection, yet paradoxically decreases the cryptococcal burden when adminstered to chronically infected rats. Enhanced expression of transforming growth factor β1 also has been detected in infected mice [61]as well as in a patient with a pulmonary nodule secondary to *C. neoformans*,[119] suggesting that transforming growth factor β1 may have a conserved role in diverse hosts.

Infection of different strains of inbred rats with the same strain of *C. neoformans* may elicit different patterns of adaptive immunity. For example, the inbred Lewis and Brown Norway strains both appear to develop granulomatous inflammation after intratracheal infection with *C. neoformans* serotype AD. Nevertheless, detailed histologic examination revealed several differences: granulomas in Lewis rats were composed of palisading epithelioid cells with central necrosis, whereas those in Brown Norway rats consisted of mature mononuclear phagocytes with occasional infiltrating eosinophils and no epithelioid transformation.[62] During the first week after infection, Lewis rats had higher expression of interferon γ and interleukin 2 but a lower level of interleukin 12, compared with the Brown Norway strain. This predominantly Th1 cytokine expression pattern in Lewis rats was associated with a significantly greater fungal burden at day 10 ($P < 0.001$), but both strains demonstrated equivalent restriction of fungal growth at later time points. Despite the predisposition of Lewis and Brown Norway rats toward Th1 or Th2 adaptive immune responses, respectively,[113] neither of these inbred strains appears to develop a definitive adaptive polarization in response to *C. neoformans* infection. This finding contrasts with those from studies in inbred mice, in which chronic fungal infection develops when the balance of cellular immunity is shifted toward a Th2 response.[53,121]

Compared with mice, the larger size of rats is advantageous for performance of experimental procedures such as intratracheal infection or sampling of CSF for characterization of the immune response in the central nervous system.[43] Unlike rabbits but similar to guinea pigs and mice, rats do not require immunosuppressive treatment to be susceptible to *C. neoformans*. A potential disadvantage of the rat model is their higher maintenance cost compared with that of mice, an important consideration for projects that require extensive breeding or long-term maintenance. Nevertheless, the future of genetic studies in the rat is bright; a variety of tools are currently available and are comparable to the excellent resources that have been developed for mice.[69,84]

Conclusions

Shortly after the discovery of *Cryptococcus* more than a century ago, the guinea pig was used as the first model host to study disease pathogenesis. Since that time, the guinea pig has largely been supplanted by several other mammalian hosts, including the rabbit, rat, and mouse, each of which have been used effectively to characterize both microbial virulence and host defense. As discussed in this review, unique characteristics of each model host confer specific advantages and disadvantages to each experimental design. For example, studies of antifungal drug therapy during meningoencephalitis have been accomplished most successfully through use of rabbit and mouse models, whereas both rats and mice have been extremely useful for analyzing the immune response during respiratory tract and disseminated disease. In general, the choice of an appropriate model will depend on the specific research objectives and the resources available to the investigator. The pace of research into host immune responses against *Cryptococcus* likely will accelerate in the foreseeable future, owing to rapid advances in genetic and genomic resources, particularly for mouse and rat models. Ongoing studies using these mammalian model hosts coupled with the substantial progress in the field of microbial genomics likely will dramatically advance our understanding and management of human cryptococcal disease.

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