Animal models: an important tool in mycology

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Animal models of fungal infections are, and will remain, a key tool in the advancement of the medical mycology. Many different types of animal models of fungal infection have been developed, with murine models the most frequently used, for studies of pathogenesis, virulence, immunology, diagnosis, and therapy. The ability to control numerous variables in performing the model allows us to mimic human disease states and quantitatively monitor the course of the disease. However, no single model can answer all questions and different animal species or different routes of infection can show somewhat different results. Thus, the choice of which animal model to use must be made carefully, addressing issues of the type of human disease to mimic, the parameters to follow and collection of the appropriate data to answer those questions being asked. This review addresses a variety of uses for animal models in medical mycology. It focuses on the most clinically important diseases affecting humans and cites various examples of the different types of studies that have been performed. Overall, animal models of fungal infection will continue to be valuable tools in addressing questions concerning fungal infections and contribute to our deeper understanding of how these infections occur, progress and can be controlled and eliminated.

Keywords fungi, animal models, antifungal drugs, host-response, fungal virulence, fungal vaccines, pathogenesis, mice, rabbits

Introduction

The use of various animals in medical, biological and microbiological research has been ongoing since the era of Pasteur and before. Many of the advances made in medicine, infectious diseases and immunology stem directly from the use of one animal species or another as a model system. Although historical precedent exists for their use, there is strong opposition to the use of laboratory animals. As a scientist in today’s society, one must be attuned to the ethics of the care and use of animals in experimental studies, follow governmental mandates and strive to determine whether or not the questions being raised can be answered by other means. Thus, scientists must answer the simple, yet elusive, question of ‘Why are animal models needed?’.

As the field of Medical Mycology progresses and matures, those scientists investigating the areas of pathogenesis, therapeutics, and immune response must determine whether in vivo studies using animal models are necessary. Why are animal models of infection performed? This question has been answered in part above, but these models provide us the means to make significant progress in the direction of ultimately understanding fungal infections, allow investigations into the evolution and progression of disease (i.e., pathogenesis), studies of what makes a particular fungus virulent and able to cause disease, aspects of...
innate and acquired immunity, how disease transmission might occur through fomites, contact or aerosols, and methods of prevention, and lastly, studies on therapeutics and diagnostics that might improve patient care and outcome.

The complexity of the host-parasite interaction cannot be mimicked by in vitro studies. Data obtained from animal experimentation aid us in demonstrating events or interactions that, up to the present time, we have no reliable alternative way to obtain. Although there are significant differences between the experimentally infected animals used in fungal models and naturally infected human beings, in numerous aspects the animal models mirror human infections remarkably well. This review will address a variety of issues including the philosophy of studying animal models, how the results derived from them are used and evaluated, the various types of models performed using different pathogenic fungi and what those studies are used for. We will document, from the work of others, but relying heavily on our own experiences, examples of engagement with these issues. Review articles are preferentially cited, as they may be used by the reader as a source for further examination of original articles, and to avoid repetition of points made in earlier reviews. Articles in English are preferentially used.

**Strengths and benefits of animal models of fungal infection**

Animal models have a number of strengths and benefits. These include mimicking clinical diseases, being predictive of clinical results, summation of in vitro data on drug activity, pharmacology, safety and drug interactions; affordability, the capacity to examine various questions rapidly and the capacity to control a multitude of variables. Animal models, particularly murine models, can be performed affordably using numbers sufficient to obtain valid statistical data, and mice (other than special congenitally or therapeutically immunocompromised mice) require less specialized animal care, support facilities and personnel.

The capacity to control different variables is the greatest benefit to performing animal models. Among the variables that can be controlled are the strains of organisms used, and increase or decrease of the inoculum size to control severity of infection. Furthermore, the choice of animal species is critical. The course of the disease in some species may mimic human disease better than others. Other animal model variables under the control of the investigator include the use of different immunosuppressive regimens to suppress particular host-cell types, choice of host and its genetics (e.g., inbred or outbred mice) and control over the route of infection to emulate different types of clinical disease. The control over the route of administration and duration of antifungal therapy when performing efficacy studies is also a benefit. Our capacity to use age and sex matched experimental animals also contributes to reproducibility. Animal models also are useful for the development of diagnostic assays. Thus, we are able to address issues in vivo that cannot be answered by in vitro tests.

**Weaknesses of animal models of fungal infection**

One also needs to be aware of the inherent weaknesses of an animal model. No single model of infection can be used to answer all questions. There can be substantial variability from one experiment to the next, which must be minimized if possible. Many murine models have the drawback of being too acute in the progression of disease, and some fungal models include the involvement of organs that are not prominent target tissues in patients. In addition, mice are difficult to use when repeated samplings of blood, for example, are desired from the same animal, if larger volumes of blood are needed. However, one may compensate for this in mice by adding extra animals to experimental groups and sacrificing cohorts at desired intervals during the experiment to obtain blood [1]. When performing therapeutic studies in animals there may be differences in drug penetration or metabolism from one species to the next and differences from that observed in humans. Lastly, as mentioned above, the model must be affordable to perform, and if the costs are too high whether in purchase price or personnel time and effort, the utility of the model is limited.

**Parameters to follow**

Because no single model can be relied on to answer the many questions one asks in experimental studies or mimic all the various diseases seen clinically, it is often necessary to use multiple models. The question in these studies becomes what parameters should be followed to obtain the best information. Two primary parameters of infection are followed, survival and fungal burden in the tissues. Survival studies result in clear data sets, but death as an end point is often not allowed by Animal Care and Use Committees, with ethical questions arising concerning the humane care and use of animals for survival studies. Many institutional committees now require euthanasia of the animals prior to death from disease. The criteria for euthanasia must be evaluated.
carefully on an individual basis, since subjective judgments can skew the data (i.e., euthanizing animals too soon makes the infection appear more severe that it truly is). Thus, criteria for euthanasia have to be as quantitative as possible, applied objectively and reproducibly to ensure accuracy, and the judgements on individual animals made without knowledge of their study group, wherever possible. Statistical evaluation of these data are appropriately done by survival analyses using a logrank test or Gehan’s Wilcoxon tests; where there are no censored data points a Wilcoxon rank sums test also can be used.

The determination of infectious burden in the target organs is another common, and sensitive, assay related to the severity of disease. For infections due to yeast (e.g., Cryptococcus, Candida, etc.) the quantification of CFU from the target organs by homogenization of the organ followed by serial dilution and plating is a useful and straightforward methodology. Infectious burden can be a sensitive parameter for studies of drug efficacy, comparative virulence or disease progression. Benefits of performing assays of infectious burden include shortened experimental durations and avoidance of survival studies. However, for hyphal organisms, like Aspergillus, the best method of infectious burden determination is controversial. Some investigators use a qPCR methodology or chitin assay, while others use CFU determinations [2–6]. Each method has benefits and drawbacks. The assay of chitin in the tissues [6] can be a tedious assay and does not indicate whether the organisms present were viable. A qPCR assay applied to the determination of Aspergillus burden [4] requires specialized equipment and reagents, and specialized sample preparation. These are drawbacks for some laboratories and commercial assay can be cost-prohibitive.

Statistical evaluation of the data obtained from determination of infectious burden is critical. A variety of statistical tests have been used by various investigators. These include parametric and nonparametric tests, including: ANOVA with or without tests used for multiple comparisons such as a Tukey’s or Student Neuman-Kuels, Kruskal-Wallis followed by a Dunn’s test and Mann-Whitney U or Wilcoxon rank sums tests. The advice of a professional statistician should be sought to determine the most appropriate statistical test for the types of experiments being done.

**Alternative models**

In the United States, The Office of Technologies Assessments (OTA) defined an alternative method as the protocol or technology that replaces the use of laboratory animals, reduces the number of animals required or refines existing procedures to minimize the level of stress produced to the animal [7].

Defining the term alternative model is difficult. Strictly, it can be defined as a model that replaces a living animal with a non-animal system. In these terms, *in vitro* systems (chemical or biological), plants, microorganisms or computer simulations should be the only alternative models used for medical research. Using a broader interpretation of the term, the use of non-vertebrates or cold-blooded animals also is considered an alternative model.

*In vitro* systems using chemical components are one of the most extensively used alternative models in medical mycology. *In vitro* tests for drug efficacy is a first step in pre-clinical trials and permits us to determine the concentrations of drug that are effective against an organism by the use of a well-defined culture media where microorganism growth is tested in the presence of differing concentrations of the drug. However, *in vitro* activity does not necessarily accurately reflect subsequent *in vivo* activity nor have the majority of antifungal drugs’ *in vitro* activity been correlated with clinical outcome. Thus, it remains necessary to determine the efficacy of new antifungal drugs using one or more of the available animal models of fungal infection prior to testing in humans.

*In vitro* systems that include biological material, i.e., cultured cells, have been used extensively in mycological research to study the interaction of the microorganism and a target tissue or specific cell-type or *vice versa*. Cell-cultures permit us to simplify an infectious event than one type of cell is present can more accurately mimic the events *in vitro*. Another more sophisticated cell culturing system is based in three-dimensional (3-D) cultures, consisting in a rotating-wall vessel that generates low turbulence, allowing cells to associate, and form 3-D structures. It will be of interest to see how the use of these types of culture systems can be incorporated into mycological research.

Although extensively used in other disciplines, the use of non-mammalian models has been minimal in mycology. The fruit fly (*Drosophila melanogaster*), the helminth, *Caenorhabditis elegans*, the amoeba, *Acathamoeba castellanii*, the slime mold, *Dictyostelium discoideum* and the Lepidoptera, *Galleria mellonella* are the non-mammalian systems that have been used in mycology. Reviews on the use of these species in medical mycology studies have been published recently.
identified using the worm *Caenorhabditis* *C. neoformans* involving pathogenesis of their natural ecological environments [16]. New genes may be relevant to interactions of the organisms in the phagocytic process, due to the similarity of the process used by mammalian macrophages, but also may be relevant to interactions of the organisms in their natural ecological environments [16]. New genes involving pathogenesis of *C. neoformans* have been identified using the worm *Caenorhabditis* as screening method [23]. Similarly, *Drosophila* and *Galleria* models of aspergillosis have been described for use in pathogenesis, host-response and therapeutic screening [24–33].

The use of these types of alternative models in mycology is in its infancy and although their use may be highly desirable, the applicability of the results to human or animal infections is for the most part unknown. How the fungi differ in behavior in the environment of the *Drosophila* or other non-mammalian system where thermal environment, organ systems and immunological processes differ significantly from those of the mammalian or avian host remains to be determined. Thus, as with the results derived from conventional animal models of fungal infection, the results derived from alternative models should be interpreted cautiously.

**Studies of pathogenesis**

*Mimicking clinical disease*

The purpose in the development of animal models in clinical mycology is to mimic, as closely as possible, the progression and the clinical signs of the infection in human patients. This allows us to understand the mechanisms involving the infectious process, host-resistance to that pathogenic process and potentially, the cure for the infection. In this section we discuss the most common animal species used in mycology as models of infection.

*Types of animals*

A first step is to choose the correct animal species, which is one that will simulate the human infection as closely as possible, or if studying veterinary fungal diseases one may actually opt to use the same animal. As described in several review papers, a variety of animals have been used in mycological research and include: mice, rats, rabbits, guinea pigs, hamsters, dogs and birds [34–43] and rarely other as toads, cats or bats [44–47].

The mouse (*Mus musculus*) is the species of choice in mycology due to its similarity to the human physiology, as well as the ease of availability, cost and other benefits discussed earlier. Both mice and humans have similarities in organ systems, biochemistries, pathologies, and similarities extend to their genomes as well. The mouse and human genome have approximately 30,000 genes encoding proteins and the proportion of genes with no homology between them is less than 1% [48]. In addition, the production of many genetically defined (i.e., inbred), genetically manipulated (e.g., gene knockouts) strains and the large numbers of immunological and genetic tools allow us to simulate and quantify what occurs during infection and be closer to human infection than models using other animal species. Mice have been used primarily as the model for systemic, pulmonary and central nervous system (CNS) infections by the most clinically important fungi.

Rabbits are the next most common species used in animal models of fungal infection. Rabbit models allow us to evaluate physical and physiological data unavailable or difficult to obtain with small mammals; for example, multiple large volumes of blood can be obtained from individual animals; serial bleeding is easier than in mice. Rabbits are also useful for the study of CNS infections because of the relative ease in obtaining high quality (i.e., without blood contamination) volumes of CSF (0.5 ml to 1 ml per sample time) from the same animal multiple times; this CSF is useful for determination of biochemical (e.g., glucose or protein) and cellular parameters (e.g., WBC counts). In contrast, the volume of CSF obtained from mice usually does not exceed 5 to 7 μl and is often a terminal procedure. The size of organs and internal structures makes possible more accurate and specific observation in rabbits than the mouse. For example, in our model of coccidioidal meningitis, the development and histological appearance of arteritis and vasculitis closely mimics that of humans [49]. Other models of CNS infection in rabbits include those using infection with *C. neoformans* [50] and *C. albicans* [51]. In addition, the use of larger animals, such as rabbits, allows for thorough clinical observations of signs and symptoms.

Rabbits have been used extensively as a model for mycoses affecting the eye due its size and similarity to the human anatomy and physiology. Experimental models of keratitis and endophthalmitis due to *A. fumigatus* [52], *C. albicans* [53–56], *C. neoformans* [57], *Histoplasma* [58], *Fusarium solani* [59,60] or *P. boydii* [61] have been developed in rabbits.

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Models of systemic fungal infection have also been described in rabbits and include *C. albicans* [62], *A. fumigatus* [63–66] and *S. prolificans* [67]. Other models including pulmonary aspergillosis [68,69], candidiasis [70,71] and cryptococcosis [72], sinusitis [73–75], endocarditis caused by *Aspergillus* [76] or *Candida* [77,78]. Rabbit models of *Aspergillus* have been reviewed in greater detail elsewhere [35,79–81].

Although rabbit models are quite useful for the study of fungal infections, and their treatment, they are more expensive to purchase, their husbandry requires special installations and careful monitoring, and greater efforts from support and animal care personnel, there are few immunological and biomolecular reagents available and there is a lack of genetically defined animals. Each of these factors needs to be considered and each can impose limits on the use of this species as an animal model for studies that includes these topics.

The next most commonly used species in medical mycology is the guinea pig. Guinea pigs are primarily used as a model for experimental cutaneous infections by *C. albicans* [82,83] *Trichophyton mentagrophytes* [84,85] and seborrheic dermatitis caused by *Malassezia* sp. [86]. In a model of tinea unguium in guinea pigs, inoculation with *T. mentagrophytes* between the toes resulted in nail collapse [87]. Guinea pigs have been used in therapeutic studies including systemic aspergillosis [88–92], cryptococcosis [93] and scedosporiosis [94]. Other models involving guinea pigs include a model of endocarditis caused by *A. fumigatus* [95], and a model of pulmonary histoplasmosis induced by aerosolized microconidia and hyphal fragments of *H. capsulatum* [96]. Guinea pigs are often used to test voriconazole (VCZ) efficacy [88,94,97–99] because, according to different authors, VCZ reaches therapeutic serum levels in this species comparable to those in humans [100–102] whereas, the drug is rapidly metabolized in mice and induces its metabolism. A drawback to the use of guinea pigs is that these animals have a complex social structure and are easily stressed under unfamiliar environments or housing conditions or the manipulations required during the course of the experimentation [103,104]. They are relatively expensive to maintain and board, and lack congenic strains.

The use of birds in models of aspergillosis deserves mention, since naturally acquired aspergillosis in the absence of immunosuppression is an important disease in avian medicine [35,105,106]. Both intravenous inoculation and pulmonary routes (intratracheal or aerosol) have been used to infect the animals, which include Japanese quail, turkeys, guinea fowl and chickens for studies of pathogenesis, therapy and vaccines [35,105]. The use of birds as a model for aspergillosis is beneficial in that therapeutics or vaccines against aspergillosis can be studied directly in the affected species rather than trying to translate results of therapy or vaccine work from rodents or other animals.

**Routes of infection**

When performing an animal model of fungal infection one must consider what route of infection to use. The various routes of infection include, pulmonary (intranasal or intratracheal), intraperitoneal, intravenous, intracranial, intrathecal or intracisternal, mucosal (vaginal or orogastric), dermal (skin or cornea) and subcutaneous. Each of these routes of infection has been used to study particular infections [34–36,38,41,107,108]. It is important to point out that animal hosts may differ in their susceptibility to challenge with the same pathogen by different routes. Our laboratory reported differences in mouse susceptibility to *Blastomyces dermatitidis* between intranasal and intraperitoneal infection. The same strain of *B. dermatitidis* at the same dose (30 CFU per animal) caused death of 50% of C3H/HeJ mice after intranasal infection vs. 17% when infection was made intraperitoneally. However, DBA/1J mice were more susceptible to intraperitoneal (83% mortality) than to intranasal infection (25% mortality). Thus, these data demonstrate that both route and mouse strain play a decisive role in modeling experimental fungal infection [109].

The majority of serious fungal infections are acquired by inhalation of infectious elements or by a breakdown of the natural body barriers i.e., skin and mucosa. Thus, in many instances a pulmonary route of infection would be optimal to initiate the model because it mimics the natural entry into the human body. For example, pulmonary inoculation with *C. neoformans* showed generalized dissemination of the fungus, with the brain and lungs heavily infected 42 days after challenge [110], mimicking human cryptococcosis. However, the development of infection and the resulting affected organs potentially can differ between animals and humans due to differential organ tropism of the infecting microorganism, the organism’s greater ability to grow in a particular organ environment or host factors. An example is substantial renal involvement in various models of aspergillosis, which is less common in human infection [35]. In light of differences in pathogenesis and organ tropism, we have the option to mimic the infectious process, which is the goal of the animal modeling, by modifying the route of infection.

Several models of systemic or localized infection have been described. Systemic infections using intravenous
inoculation are used often in models of infection to reproduce in the animal a mimic of human dissemination, independent of the natural entry of the pathogen on the host. Systemic infections inoculating a suspension containing different fungal elements intravenously have been tested with the most common pathogenic fungi. Systemic infections of Aspergillus spp. [3], Cryptococcus spp. [111], Candida spp. [35,36,38,107], Coccidioides immitis [34,39], Histoplasma capsulatum [34,39], Scedosporium spp. [98,112,113], Sporothrix schenckii [114], Paracoccidioides brasiliensis [115], Penicillium marneffei [116], Hansenula anomala [117], Fusarium sp. [118] or Trichosporon asahii [97] have been described in the literature using different mammalian species. The intravenous route depends on anatomic characteristics of the animal species. In mice or rats systemic infection is performed most often by using the lateral tail veins. However, in other animals where tail veins are not easily accessible or do not exist, other routes are used. The lateral ear vein and the penile vein are the choose routes for infection in rabbits and guinea pigs, respectively.

Localized or site-specific infections have been developed over the years of animal modeling to simulate the pathology in the target organs associated with one site of infection in humans. With this purpose, pulmonary, brain, intraperitoneal, ocular, superficial, vaginal or subcutaneous models have been developed.

CNS is a common site of hematogenous dissemination of fungi such as A. fumigatus, C. neoformans C. immitis, Scedosporium prolificans and some phaeohyphomycetes in humans and infection there can result in high mortality. Mimicking the natural pulmonary establishment of a pathogen does not always result in dissemination to the CNS in animals. In the case of cerebral aspergillosis direct intracranial inoculation of conidia results in high tissue burdens in the brain, with the histopathological lesions and cellular host-response similar to the observations in human infections involving the CNS, although this is not the natural route by which humans acquire the infection [119].

Different techniques such as the intracerebral (or intracranial), intrathecal or intracisternal inoculation are available for the study of fungal infections associated with the CNS. Intracranial infection is performed by direct inoculation in the brain parenchyma through the midline of the cranium in mice [120]. However, as mice mature the sutures between the frontal and parietal plates fuse, making the bregma more difficult to locate. Thus, injecting through the fontanelle is useful only for mice up to about 8-weeks of age. Although infection spreads to other organs, CNS fungal burdens remain higher than in other organs [121,122]. In the example of aspergillosis, hyphal growth and development of abscesses and necrotic areas mimic the common complications observed in clinical cases of cerebral aspergillosis [119,123]. Intracerebral models of infection also have been used to test therapeutic options for treatment of aspergillosis, coccidioidomycosis, scedosporiosis, histoplasmosis and cryptococcosis [121,122,124–128]. Another route of infection to establish CNS infection in mice is intrathecal inoculation, which has been used in a model of coccidioidomycosis to simulate the meningitis and vasculitis associated with CNS complications caused by hematogenous dissemination of the fungus in human disease [129]. The technique requires a skin incision over the lumbar vertebrae and inoculation of an arthrocondrial, spherule or endospore suspension into the subarachnoid space via lumbar puncture [129]. Via this route of infection, the fungus can be recovered from brain and meninges as early as three days after infection, and by 8 days postinfection, the histopathologic abnormalities found in the brain or spinal cord include acute meningitis, vasculitis and polymorphonuclear cells infiltrates [129]. Similar to the intracerebral route of infection, extrameningeal dissemination is observed; however, CNS involvement is greater than that reached in systemic or pulmonary infections [129]. Intracisternal inoculation for the establishment of infection in the CNS has been used as route of infection in rabbit models of meningitis by C. neoformans and C. immitis [49,50].

Pulmonary diseases can be initiated using different techniques. The most common technique is by intranasal instillation of a fungal suspension. However, the number of fungal elements arriving in lung tissue can be uncertain, depending on the anesthesia used and experience of the person doing the procedure. In early studies, our laboratory found that, using a standardized procedure, 1 h after intranasal inoculation with B. dermatitidis yeasts, 33.5% of the inoculum CFUs could be routinely recovered from lungs, and results in a consistent model of pulmonary blastomycosis [130]. This technique is easy to perform and several reproducible models of fungal infection have been established including pulmonary aspergillosis [3,35], cryptococcosis [131], histoplasmosis [34,39,46], blastomycosis [34,39], paracoccidioidomycosis [39] and zygomycosis [38,75,132] in different mammalian species. Some investigators prefer to use inhalation of dry fungal conidia as the method of pulmonary infection. A comparative study using titanium dioxide particles showed that inhalation results in a more homogenous distribution of the particles in lungs than does the instillation technique [133]. In another study, delivery
by inhalation was compared to intranasal instillation for the establishment of pulmonary aspergillosis in rats; higher fungal burdens with smaller standard deviation and more homogenous pneumonia was reported in animals infected by inhalation than by instillation [108]. The use of aerosol chambers reproduces, with the exception of higher numbers of fungal conidia inhaled, the natural entry of the microorganism in the respiratory tract. Separate studies have demonstrated the reproducibility of delivering *A. fumigatus* conidia to the lungs using inhalation [108,134,135]. The third method used for inoculation for pulmonary infection is the intratracheal route. Although better for the consistency of inoculum administration, it requires a minor surgical procedure. An incision needs to be made and organisms injected directly into the trachea. Various models using this method have been reported such as those using *Penicillium marneffei* [116], and *C. albicans* [70,71], as well as others [35,36,38,39].

Mucosal sites of infection include oral, gastrointestinal tract and vaginal and have been primarily in rodents for studies of *C. albicans* [136,137]. Establishment of gastrointestinal infection is done by gavage with a suspension of the yeast, allowing the animals to drink from a suspension of yeast or by feeding the fungus in pellets. Immunocompetent, immunosuppressed, congenitally immunodeficient, germ-free and antibiotic-treated mice have all been used in this type of model [137]. In addition, adult and infant mice have been used [136,138]. The model can be manipulated to mimic different patient populations at risk for development of mucosal candidiasis by altering the immunosuppressive regimen or changing the strain of mouse used. Several studies have used neutropenic mice to examine *Candida* translocation from the gut as a source of disseminated disease [38,137,139–143], a syndrome that occurs in neutropenic and chemotherapy patients. In contrast, AIDS patient develop mucocutaneous candidiasis, but the yeast do not disseminate. Orogastrointestinal candidiasis in SCID mice shows a similar lack of dissemination from the gut and thus provides a good model for studies of therapy and host-interaction [144,145].

Studies on vaginal candidiasis are also done in rodents and primarily in mice, although other species have been used [38,146–148]. Infection of animals is easily done by lavaging a suspension of yeast into the vagina. To establish and maintain vaginitis in mice it is a requisite that estrogen be administered to induce estrus [38,149]. Interestingly, not all mouse strains are equally responsive to the effects of exogenous estrogen treatment, with CD-1 mice being particularly resistant to estrogenization [150]. This resistance to estrogenization carries over to resistance to the establishment of experimental vaginal candidiasis [151,152]. Thus, choice of mouse strain can be critical to the success of the model. The use of the vaginal model has included pathogenesis, therapeutic and host-response studies [38,146,153–158].

**Organ specificity**

As mentioned before, when performing a model of fungal infection one must be aware that each fungus may preferentially grow in a particular organ system in the animal and these may not be the same as the primary tissues infected in humans. These tropisms may be apparent regardless of the route of infection or can also be determined by the route of infection as discussed above.

For example, in most murine models of candidiasis using outbred or inbred mice infected intravenously, the kidney is the primary organ infected, with lungs, brain, liver and spleen infected more transiently [159]. Although kidney infection is not so prominent in humans, renal infection in mice does mimic the course of sepsis in humans [1]. However, the use of a genetically deficient mouse strain, beige mice, shows a change in tissue tropism with the liver and spleens having progressive disease [160].

Infection of mice with *C. neoformans* provides an excellent example of a fungal model that demonstrates the same organ involvement, CNS, as is found in humans. Although the reasons are not entirely elucidated, *C. neoformans* requires exogenous substrates to synthesize melanin, and some suggest than the tropism of *C. neoformans* for the brain could be due to its capacity to make melanin from catecholamines. Observations showing that areas of the brain rich in catecholamines are frequently infected by *C. neoformans* support this suggestion. Recently, we have reported differences in organ involvement between *C. gattii* and *C. neoformans var. grubii* in a systemic model of cryptococcosis in BALB/c mice [161]. Infection with *C. grubii* showed a temporal increase of CFU in brain and liver but no yeast were recovered from the brain of animals infected with *C. gattii*. Interestingly, and reported for first time, long-term infection with *C. gattii* resulted in unusual skin and intestinal mucosal lesions in hydrocortisone-suppressed and in immunocompetent BALB/c mice, whereas these types of lesions were not found in *C. grubii* infected mice [161].

Murine models of blastomycosis provide examples for tissue involvement based on routes of infection. In humans, primary pulmonary disease with dissemination occurs; cutaneous lesions are common sites of
disseminated disease. In mice infected intranasally with *B. dermatitidis*, pulmonary disease is quite severe and lethal [39,41,130]. Although the organism does not disseminate from the lungs to other tissues in the acute infection produced in the mouse, cutaneous blastomycosis is mimicked by using subcutaneous inoculation as the route of infection [39,41].

**Virulence studies**

Historically, the comparative virulence of strains of fungi (e.g., a mutant and the parent, or multiple isolates of the same species) has been studied in these models and comparisons made based on lethality or fungal burden in the target tissues. Animal modeling is a useful method to determine the severity of the experimental infection as a correlate of the virulence of the infecting fungus. Severity is primarily evaluated by mortality and quantitative recovery of the fungus in affected organs, while histopathology of affected tissues and the host response give us a picture of the affected organs, while histopathology of affected tissues and the host response give us a picture of the interaction between host and pathogen *in vivo*. Understanding the mechanisms regulating fungal virulence and the interaction with the host is necessary for a better understanding of the infectious process. The ability of the fungus to survive, grow, and evade the immune system in mammalian tissues seems to be a strain attribute that depends on the expression and regulation of various genes (e.g., virulence factors).

Classically, virulence factors have been considered to be those traits that are present in the microorganism that are needed to cause disease and when absent, the microorganism loses its ability to produce disease, but the loss of the virulence factors does not alter the ability of the organism to grow under other conditions. In bacteria, adhesion, colonization, invasion, immune-response evasion, capsule formation and toxin release are strongly related to virulence. Although capsule formation by *Cryptococcus* spp. is clearly a virulence factor [162], other factors, responsible for virulence in fungi, are not as well defined.

Because most fungi have a free living saprobic phase, not requiring a host to complete its biological cycle, it would seem that infection is merely an accidental encounter between the fungus and the host. As a consequence, we can postulate that fungal physiology has developed as a result of a natural selection outside of human tissues, and the mechanisms that give the fungus the ability to cause infection may be cross-linked to the necessity of survival, in the environment.

For example, melanin is a hydrophobic pigment present in several species of pathogenic fungi [163,164]. Melanin seems to play an important role as a virulence factor and contributes to the severity of the disease. Nonpigmented strains of *C. neoformans*, generated by UV spontaneous mutation, were shown less virulent than pigmented strains in animal models, but the mutation did not result in avirulence [165]. Similarly, melanin-deficient *Wangiella dermatitidis* [166,167] and *A. fumigatus* [168,169] showed loss of virulence in comparison to their respective parental wild-types in murine models. Important roles have been attributed to the melanin as a virulence factor, contributing protecting the organism from oxidative stress and macrophage digestion in mammalian tissues and giving higher resistance to antifungal agents [170,171].

**Strains of fungi**

Studies of comparative virulence among isolates of a fungal species have shown, for many organisms, that when inoculated into an animal in equal numbers of organisms, some isolates are more pathogenic than are others. Differences among these may be large or relatively small depending on the organism. Thus, it is crucial to determine the relative virulence of the fungal strain under study prior to embarking on additional studies looking for virulence factors or pathogenic mechanisms. Studies on virulence and host-resistance most often are done using one strain of fungus and several strains of mice or several strains of fungus and one strain of mice. An example of how this approach can be troublesome comes from our experience with studies on the pathogenicity of *Saccharomyces cerevisiae*. Using a murine model studied in CD-1 mice we surveyed 28 clinical and nonclinical isolates of *S. cerevisiae* for their capacity to proliferate and persist in the brain [172]. A continuum of virulence was found ranging from those able to proliferate by about 5-fold to those that were rapidly cleared from the tissues [172]. In a subsequent set of studies we examined these same isolates in a different strain of mouse, DBA/2, and found that some of the isolates were lethal (increase in virulence), but that the rank-order of virulence was not the same in DBA/2 mice as it was in CD-1 mice [173]. These differences indicate the potential for multiple strategies of virulence [173]. Thus, one should not assume that because an organism appears virulent or nonvirulent in one model that it will appear the same way when used in a different model of infection.

**Molecular tools and fungal gene knockouts**

With the advent of molecular biological techniques, studies of virulence factors have routinely used a ‘gene knockout’ approach, where one or more genes are removed or inactivated or replaced and the virulence of...
this strain is compared with the wild-type parental strain [174]. Numerous studies have been done to identify virulence factors and mechanisms that mediate the pathogenesis of the fungi and have been presented in detail elsewhere [3,9,35,41,75,115,163,164,175–186]. Examples of these types of studies follow. Filamentous growth of C. albicans is important in establishing mucosal infection [187]. Disruption of genes involved in filamentation resulted in decreased virulence in animal models. Mutation of int1, a gene involved in filamentation and adherence in C. albicans [188] showed reduced mortality in a systemic murine model, but fungal burden demonstrated higher persistence in kidneys than did the wild-type strain [189]. Cph1 and efg1 are transcriptional regulators in controlling filamentous growth in C. albicans that activate different pathways involving filamentation. Efg1 seems to drive the major pathway for filamentation and cph1 has a lesser role. However single mutants for each of these regulators and double mutants still showed capacity to form filaments as a function of the medium used, suggesting that additional genes are involved in filamentation [190]. Double cph1 and efg1 mutants were found to be avirulent in a mouse model [191].

Several genes and proteins of A. fumigatus have been also been linked to pathogenesis. Genes involved with cell wall integrity, evasion of the immune response, toxins and extracellular enzymes related to direct attack of the host tissues have been recently and extensively reviewed [192]. Aspergillus strains defective in particular genes involved may demonstrate a reduction of virulence, but can still retain pathogenic capability. For instance, double chitin synthase mutants (chsC-1chsG-) showed reduced chitin synthase activity (required for cell wall assembly), as well as anomalous growth in A. fumigatus. However, the double mutant, despite showing reduced pathogenicity in comparison to the wild-type strain, still caused pulmonary disease in a neutropenic mouse [193]. Similar results were found in chsE mutants [194]. Deletions of rhaA, fos-1 and pksP genes also show reduction of virulence in models of murine aspergillosis [195–197]. Histidine kinases [198,199], and calcineurin [200–202] serve as additional examples of genes linked to virulence by studies done in animal models.

As should be evident, using the approach of studying single genes and their impact on virulence is a slow and tedious process, requiring many animals. More recent advances in the study of virulence factors have taken other approaches. One method to assess virulence is that of in vivo gene expression and there are two related methodologies for gene-profiling of fungi during infection that use reporter genes. These are in vivo expression technology (IVET) or constructing fusion proteins tagged with green fluorescent protein (reviewed in [203,204]). IVET has been successfully used for C. albicans [205] and Histoplasma capsulatum [206]. In Candida albicans IVET demonstrated that SAP2 was expressed during systemic, but not mucosal murine infection. For A. fumigatus, Langfelder et al. [207] formed a green fluorescent protein (GFP) fusion with polyketide synthase (pksP-egfp) and followed the in vivo expression of this GFP-fusion protein during pulmonary infection in corticosteroid-suppressed mice. They showed expression of pksP in the hyphae of germinating conidia in the lungs of infected animals.

A second method of assessing virulence is that of inoculating animals with a pool of mutants and letting the mouse eliminate those that are not virulent. In studies on S. cerevisiae, pools of isogenic strains were used to infect mice to determine genes important to survival in vivo by determining which of the isogenic strains was recovered from the animals [208]. Signature Tagged Mutagenesis (SMT) has been applied successfully to the study of in vivo gene expression of Cryptococcus [209] and A. fumigatus [210] in murine models. This methodology uses a transposon mutagenesis system in which each transposon mutant is tagged with a unique DNA sequence that is to be followed using PCR and Southern blotting. To examine in vivo expression, a pool of individually tagged mutants is prepared and inoculated into mice and organisms recovered at various times postinfection. In the Aspergillus studies pools consisted of 84 or 96 unique mutants, which increases the number of mutants that can be screened in one experiment [210]. The use of this methodology for A. fumigatus resulted in the identification of PABA synthase as an essential gene for virulence [210]. Similarly, the Cryptococcus work resulted in the identification of 5 strains with reduced virulence and one strain with increased virulence [209].

Lastly, in vivo gene profiling is a desirable method to use for the detection of genes potentially relevant to virulence. Comparisons of A. fumigatus transcription in vivo, during pulmonary infection of mice, to in vitro transcription, demonstrated higher transcription of rhaA, a RAS-related protein and fos-1, a gene encoding histidine kinase protein in vivo than in vitro. Interestingly, expression of pksP related to conidial pigmentation was only detected in pulmonary infection, but was not detected in vitro [211]. In vivo gene expression has also been examined by serial analysis of gene expression (SAGE) technology in elegant studies done on C. neoformans in a rabbit model of meningitis [212]. Generation of sequence tags (ca. 14 bp) for each cryptococcal gene allowed for RNA expression of the
organism to be followed. The results of the study indicated that *C. neoformans* *in vivo* showed over 300 highly expressed sequence tags (i.e., genes), involved in a variety of cellular functions [212] and will allow more focused efforts on particular pathways and gene sets.

**Studies of host response**

Infection can be considered as an imbalance between the host-defenses and the infectious agent, with the host unable to control the proliferation of the infectious agent. How the host is able to fend off fungal infections or why the host is susceptible to fungal infections is an area of intense study, for which animal models of infection have proven invaluable. Historically the use of animal models, and particularly murine models, has been crucial to our understanding of host-response to fungal infection. The greater susceptibility of immunosuppressed animals to the mycoses, the increased refractoriness of immunosuppressed animals to antifungal therapy, and, in aspergillosis, the requirement for immunosuppression in order for progressive invasive pulmonary disease to develop, corroborate findings in clinical medicine. Various methodologies and strains of mice have been used to correlate the importance of various cell types (e.g., PMNs, macrophages, different lymphocyte populations, antibodies, etc.) [39,213,214]. Good examples of these studies are the use of spontaneously occurring mutations in mouse strains such as athymic nude mice, depletion of particular cell populations or cytokines by antibody treatment (e.g., anti-PMN, anti-mu, or anti-interferon-γ) or other means of cell depletion like silica or carageenan treatment or immunosuppressive cytotoxic regimens [39,213]. Much progress has been made with the advent of site-directed mutagenesis for generation of cytokine or receptor deficient strains of mice, cloning and availability of specific cytokines for use *in vitro* or *in vivo* and in the methods that can be used to detect and follow the global genesis of the host’s response to infection. These methodologies continue to reveal to us the complicated nature of the host-parasite interaction. Given the vast number of articles on these subjects, we will only present a brief overview addressing more recent studies and use examples of how animal models of fungal infection have been used to move the field forward.

How does the host respond during a fungal infection and which aspects of the immunological response are most important? This question is multifaceted and quite complex, and as yet not fully answered as the host-response can be tissue specific and organism specific. Animal models provide us the means with which to address different issues.

**Laboratory manipulations**

As the reader will remember from the introductory remarks, no single model can be used to answer all questions. Therefore, the investigator may need to perform a manipulation of the animals to address a particular immunological question. These manipulations include, but are not limited to administration of an immunosuppressive regimen (e.g., cytotoxic chemotherapy or glucocorticoids) given alone or in combination with antibiotic regimens to prevent secondary bacterial infections, antibodies to interfere with or inactivate a cell population (e.g., anti-PMN) or particular cytokine (e.g., anti-IL-12), administration of a chemical or drug to induce a metabolic change (e.g., streptozocin to induce type II diabetes or estrogen to induce estrus), surgical alteration of animals (e.g., thymectomy, orchidectomy, or ovariectomy), gamma-irradiation, and changes in diet (e.g., amino acid deficiency). Each of these manipulations affects the host-response to infection and those effects should be defined. For example, in recent studies on orogastrointestinal candidiasis we found that administration of 5-fluorouracil resulted in dissemination of *C. albicans* from the gut tissues to visceral organs, nicely emulating a clinical situation of the cancer chemotherapy patient that develops systemic candidiasis [139]. However, during our preliminary studies we noted that mice were succumbing to secondary bacterial infection rather than fungal disease and found it necessary to include a combination regimen of oral and parenteral antibacterials to allow for the development of the candidal infection [139].

**Genetic manipulations**

Most useful for studies of host response to fungal infections are animals, particularly mice, with defined genetic defects in their immune systems. Animals of this type include T-cell deficient nude mice, beige mice with phagocytic cell degranulation defects, chronic granulomatous disease mice with defects in generating reactive oxygen species by phagocytes, and SCID mice with T and B cell defects. Each of these types of animals has been used in studies of fungal infection examining host-response [35,36,38,39,107,215,216]. However, a drawback to these types of animals is that often the mutation responsible for the defect has pleiotropic effects, which may or may not influence the results. Although nude mice have no mature T cells, their macrophages have been described as being at a higher
basal state of activation and they have increased numbers of NK cells [217] and have, for example, shown paradoxical resistance early during infection with C. albicans [218].

More desirable and now possible with the advent of modern molecular techniques is the generation of gene knockout (KO) mice, which have one or more specific genes inactivated or deleted. The commercial availability of these animals, although quite expensive to purchase, makes a variety of studies possible. Several of these strains of animals have been mentioned already, but include KO for a specific cytokine, enzyme, cell receptor, etc. These types of mice have become invaluable for determining the role played by a particular molecule in host response [177,214,219]. Possibly confounding effects can occur because of redundancy in the immune system (more than one gene may control a defense pathway), or compensatory hyperactivity (up-regulation of gene expression to compensate for the gene knocked out).

Genetic susceptibility to fungal infection in humans is a growing field of study [220], and it benefits from observations of genetic susceptibility in mice [150,173,221–224]. In addition, the continued accumulation of haplotype polymorphisms into databases for mouse strains allows investigators to further refine their searches for genes or gene families involved in susceptibility [220]. Furthermore, the advent of molecular manipulation has allowed for the insertion of human genes into mice resulting in a transgenic humanized mouse. For example CD13 insertion has been studied in relationship to the pathogenicity of human coronavirus in mice [225]. These types of studies further refine and extend the potential of murine models for the study of disease, since they permit study of human-specific molecules and/or cells in an in vivo environment. Future studies using transgenic mice humanized by the insertion of a single human gene (e.g., cytokine or chemokine receptor molecules, Toll-like receptors, etc) will likely be applied to fungal diseases and will undoubtedly provide extremely interesting results.

**Cytokine expression**

As we have come to learn over the last 30 years, cytokines are the primary cellular signals responsible for the activation and modulation of the primary innate cellular host-response, as well as the genesis and development of the adaptive response to infection. The expression of particular cytokines signal whether the host-response will be protective or nonprotective as they drive it towards a Th1 or Th2 response. Studies on the genesis of host response can be greatly aided by examination of the cytokine response in vivo [214,219,226–229]. Animal models provide the possibility of examining specific tissues or organs for cytokine expression and the availability of immunological reagents for mice and variety of strains of mice make them ideal for these studies. Methodologies include ELISA assays for cytokine proteins, cytokine protein macroarrays, RT-PCR assays for individual cytokines and mouse DNA microarray for use in examining the global host-response to infection. The use of other animals as models for studies of host-response is more limited due to the severely limited availability of immunological reagents and genetically defined animals.

Examples of studies examining cytokine expression include infection with Aspergillus, Candida, Cryptococcus, Coccidioides, Histoplasma, Paracoccidioides, and Pneumocystis [230–244]. Each of these used a mouse model. In contrast, examination of cytokine expression in rabbit models of fungal infection is extremely limited due to a lack of reagents. Using a rabbit model of meningeal coccidioidomycosis our group has examined the temporal expression of mRNA for several cytokines in the basilar artery. We accomplished this by developing our own primers for sequenced rabbit cytokines and used PCR methodology to demonstrate up-regulation of mRNA encoding for different proinflammatory interleukins, as well as MCP-1, iNOS and MMP-9 in the brain basilar artery [245]. The rabbit will become an even more useful animal for modeling as additional immunological reagents become available and research is done on the immune response of the rabbit.

**Innate and adaptive immunity: genesis and regulation of the immune response**

Innate immunity is phylogenetically the oldest mechanism of defense and is present in all multicellular organisms [246]. Soluble proteins made by the host play an important role in innate immunity to fungal infection. The activation of complement promotes inflammation via chemotactic subunits as well as opsonization of fungi [247–249]; several fungi activate complement through the alternate pathway [247–249]. Studies in murine models have shown the importance of complement controlling infection by C. albicans. DBA/2 mice, which are defective in C5, are extremely sensitive to systemic candidiasis and aspergillosis [221,222]. Infusion of C5-sufficient serum into DBA/2 mice infected systemically with C. albicans increased survival of the animals, reduced tissue burdens of fungus and reduced the severity of lesions in the
The cellular receptor TLR family of proteins recognizes microbe-derived molecules and stimulates the innate immune response by inducing the production of proinflammatory cytokines. The inflammatory response is induced after binding of a TLR with its ligand, which subsequently induces, such as through the MYD88-dependent pathway, transcriptional regulation of TNF-α through the activation of the NF-κB pathway in macrophages and dendritic cells [26,214,219]. Since this family of proteins was first described in Drosophila sp., 10 different TLR have been identified in mammals based on sequence homology to the Drosophila Toll protein [274]. TLR-4 and TLR-2 contribute strongly in signaling responses to A. fumigatus and C. albicans, as studies relating deficiencies in TLRs to severity of disease have shown [26,275–277].

Experimentally, peritoneal and alveolar macrophages from TLR-2−/− mice showed a significant decrease in TNF-α production compared to naïve cells following A. fumigatus infection [24]. Experimental aspergillosis in TLR2 knockout mice resulted also in low production of TNF-α and IL-12, as well as reduced survival and higher fungal burdens in the tissues than competent mice [24]. Blease et al. reported that TLRs could be regulated by circulating interleukins, when IL-18 defective mice infected with pulmonary A. fumigatus showed a down-regulation of TLR-2 mRNA in comparison to that from IL-18 competent animals [278]. Regulatory processes acting on TLRs play an important role in orchestrating an adequate innate response against specific pathogens, introducing a new concept about ‘specificity’ of the innate response [279,280].

Natural killer (NK) cells are a population of large granular lymphocytes present in non-immune animals and are well known for their capacity to kill tumor cells by cytotoxic mechanisms. NK have also been proposed to play a role in innate immunity against virus, bacteria, parasites and fungi [281]. Cell wall components from C. neoformans and C. albicans increase NK activation [282–284]. Murphy et al. demonstrated that NK recognize cell wall components of C. neoformans and made contact with the microorganism by microvilli, demonstrating differences in the binding mechanisms used by NK for C. neoformans and tumor targets [284]. Inhibition of NK by anti-NK antiserum increased the CFU of C. neoformans in lungs of mice after systemic infection in comparison to the control animals but not in liver, kidneys or brain. However, no differences were reported when the intrathecal route of infection was used [285]. From later murine experiments it appears that NK cells potentiate macrophage anticytotoxic activity by production of IFN-γ rather than by direct killing [286,287].
Lymphocyte T Helper (Th) cells are subsets of the CD4⁺ T cell lineage that modulate the immune response by cytokine release. From the CD4⁺ subsets, Th1 and Th2, differentiation of Th1 cells is the predominant response to infections by invasive fungi and Th2 in allergic responses to inhaled fungi on mucosal surfaces [288]. Although these patterns of response are constant in mammals, there are significant differences between human and, for example, mouse immunology that must be taken into account. Differences in TLR expression, cytokine secretion, Th1/Th2 differentiation, and antigen-presenting functions, among others, have been extensively reported and should be taken into account to interpret data obtained from animal modeling [214].

Classically, cytokine release by Th1 cells has been known as pro-inflammatory cytokines, because of their roles in enhancing macrophage activation and promoting inflammation. Activated macrophages and dendritic cells induce Th1 cell development through a STAT4-dependent pathway by major release of IL-12. The major effector function of Th1 cells is to promote activation of macrophages by IFN-γ. The use of IL-12 knockout mice has shown them to be highly sensitive to C. albicans, H. capsulatum, C. neoformans and C. immitis infection, demonstrating the importance of this cytokine to resistance to these organisms [289–293]. However, the up-regulation of this cytokine can be also responsible for an uncontrolled inflammatory process that affects the host tissues. It has been reported that administration of exogenous IL-12 induces a less severe infection by P. brasiliensis in mice, but increases the severity of inflammation [294].

In contrast to IL-12, IL-10, secreted by Th2 cells, plays a role in inhibiting the inflammatory response mediated by Th1 cells and also promotes the Th2 response [295,296]. Down-regulation of Th1 response results in diminution of resistance to various fungal pathogens. Data from animal models support this inflammatory interaction, since IL-10 KO mice have been shown more resistant than control wild-type mice to infection by clinically important fungi [234,297–299]. It has been reported that neutralization of IL-10 up-regulates production of nitric oxide, contributing to an effective fungicidal effect against systemic candidiasis [300]. However, a reported pivotal function of IL-10 shows the flexibility of the immune response and its regulation. For example, physiological levels of IL-10 were required for Th1 development in IL-12 KO mice indicating the relevance of a finely regulated balance in cytokine expression [301].

Although Th1 cells are viewed as necessary in defending the host against acute fungal infections, Th2 cytokines principally down-regulate the inflammatory response. In systemic aspergillosis in mice, administration of exogenous administration of IL-12 failed to induce resistance, whereas IL-4 cured 70% of the mice and protected them from a second lethal challenge [235]. Stimulation of IgE production and eosinophil activation by Th2 cytokines are responsible for the prolonged immune response and the hyper-reactivity reported in allergic aspergillosis [302,303]. Recent studies suggest members of the IL-17 cytokine family as regulators of the Th2 response instead of Th1 in pulmonary aspergillosis [304].

The function of T cells is clear against fungal infections, but the role of B cells in protection from most fungal infections is less certain. Recent studies showed the importance of B cells in regulating the severity of systemic cryptococcosis. B cell defective mice showed greater susceptibility to systemic and pulmonary infection than did C57BL/6J mice suggesting a protective B response. However, transferring IgG to B-cell defective animals did not improve the course of the infection, but did in C57BL/6J. In addition, higher levels of IFN-γ, MCP1 and MIP-1α and lower levels of IL-12 were found in B cell defective mice than in C57BL/6J animals after infection [305]. These observations may be due to an over-exuberant Th1 response in response to the infection and suggest immunomodulatory effects of B cells and antibodies [305,306]. Similarly, Magee et al. demonstrated by using microarray technology, up-regulation of B-cell related genes in mice immunized with formalin-killed spherules, and that immunization of B-cell deficient MuMT mice, resulted in no protection against coccidioidomycosis, which supports the protective role of B cells [307].

Although the innate immune response is a primary host-defense mechanism, long-lasting resistance to re-infection due to acquired immunity is also very important [214,219,226]. Adaptive immunity is the result of long-lived memory CD4 and CD8 T cells, which upon restimulation with a specific antigen initiate the inflammatory immune response, phagocytic cell activation and enhancement of cellular mechanisms of killing. There is strong evidence that infection and recovery from infection with various systemic fungi, Histoplasma, Coccidioides, Blastomyces and Paracoccidioides, corroborated in animal models, lead to life-long immunity [214,219,226]. In contrast, infection and recovery from disease due to an organism such as Candida does not appear to lead to life-long immunity and re-infection can occur (e.g., recurrent vaginitis).
Modulation of host response

There are a number of different methods used to modulate the host response. The inflammatory response can be down-regulated with glucocorticoids, inhibitors of TNF-α such as pentoxifylline [308], antibodies to specific cytokines or cytotoxic chemotherapies. Up-regulation or stimulation of the immune response can be done by administration of specific cytokines (e.g., TNF-α, IFN-γ, IL-12, CSF’s), specific antigen administration, and nonspecific stimulators such as alum, BCG, muramyl dipeptide, CpG DNAs, monophosphoryl lipids and saponins. The latter are used most often as adjuvants in vaccine preparations [309].

A primary focus of immuno-modulation of fungal infections has been in therapy using particular cytokines as adjunct to conventional therapy [107,227,228,310–314]. Administration of exogenous IFN-γ to mice has proven effective in cryptococcosis, histoplasmosis, paracoccidioidomycosis, candidiasis, and aspergillosis [115,315–324]. Similarly, IL-12 and IL-18 stimulate mice to be more resistant to cryptococcosis and histoplasmosis [287,293,325–333].

Colony stimulating factors (CSFs) have also been used as adjunctive stimulators of the host response [107,213,227,228,310,311]. For example, efforts to reverse neutropenia or enhance PMN production in normal mice by exogenous administration of G-CSF have failed in different models of systemic fungal infection. G-CSF in combination with antifungal therapy showed no advantage in comparison to the antifungal treatment alone in murine models of scedosporiosis [334], mucosal and intra-abdominal candidiasis [144,335] or meningal cryptococcosis [336]. Similar results have been observed with GM-CSF and M-CSF. However, successful enhancement of antifungal therapy against candidiasis [310,337,338], histoplasmosis [339] and aspergillosis [340,341] in mice by G-CSF has been reported.

Vaccines

A long-term goal in the field of medical mycology has been the development of preventative vaccines. Approaches using strains defective in virulence or killed organisms in different forms, i.e., conidia, hyphae or yeast, have shown the capacity to induce at least partial protection of animals from many common fungal pathogens [342–347].

Early studies showed that sub-lethal doses of A. fumigatus or viable avirulent and acapsular strains of C. neoformans followed by lethal challenge of A. fumigatus or C. neoformans, respectively, protected animals from a lethal challenge [348–350]. In the 1960s Levine et al. demonstrated that injections of formalin killed spherules (FKS) of C. immitis protected mice from pulmonary infection [351–353]. However, clinical trials done in 2,867 subjects showed no significant protection in subjects that received three intramuscular injections (1.75 mg of FKS each) over the placebo group [354]. Unfortunately, the high incidence of severe reaction at the site of injection precluded future studies using doses equivalent to those used in the animal studies.

Today, the focus of vaccine research is on specific antigens and several have shown protective effects in laboratory animals against different fungal infections. The efforts to develop an effective vaccine against coccidioidomycosis continue, with several different specific proteins and preparations having shown promise in murine studies [355,356]. A respiratory protein, identified as β-1,3-glucanosyltransferase (Gel1), confers protection against pulmonary coccidioidomycosis by C. posadasii in mice; there was an increase of IL-12 and IFN-γ as well as IgG in mice receiving rGel1, demonstrating the importance of the Th1 response in protection against this dimorphic fungus [357,358]. Proline-rich antigen (PRA), also called Antigen 2 (Ag2), and a glycoprotein of the lipid-rich outer wall (SOWgp) layer from C. immitis have also demonstrated protective properties by enhancing Th1 response in animal models [359,360].

The use of Th1 stimulators has demonstrated an increase in the protective effect of vaccines in animal models. For example, unmethylated CpG oligonucleotides in combination with A. fumigatus allergens [361], or antigens from C. immitis [362] stimulated Th1 response increasing resistance. However, the use of CpG increased the susceptibility of mice to challenge with C. albicans [363]. These contradictory effects on the use of CpG tell us about the complexity of general immunity.

Other evidence about the activation of acquired responses against a fungus and production of specific antibodies and the importance they may play in coordinating immunity exists [364]. Detection of specific antibodies, used for diagnostic and epidemiologic purposes [365–368], supports this role. Mannans and glucuronoxylomannan that stimulate protective and nonprotective IgM production have been associated with protection against C. albicans and C. neoformans, respectively [369,370], suggesting that individual epitopes differ in their ability to elicit protective antibody. Immunizations with liposomal encapsulated adhesin from C. albicans protected BALB/c mice against systemic candidiasis and polyclonal anti-serum from the immunized mice protected naïve BALB/c and SCID
mice [369]. Because SCID mice are defective in B and T cells, these cells appear not to be involved in the protection. The authors suggest that protection could be transferred by antibody that agglutinates C. albicans cells [369]. Similarly, mice immunized with capsular glucuronoxylomannan covalently bound to tetanus toxoid conferred protection against C. neoformans, and antibody titer during the early phase of infection correlated with clearance of the organisms [371]. The true potential of antibodies may have been recently demonstrated by studies showing that a laminarin-diphtheria toxoid conjugate prolonged survival of mice infected with C. albicans or A. fumigatus and that transfer of whole immunogenic serum to naïve animals resulted in immunoprotective properties ascribed to IgM and IgG1 [372]. One may hope that this type of study could lead to the goal of having a Universal Vaccine against clinically important fungi.

Interestingly, acquired immunity may be tissue specific and not translate into other organs. In a murine model of vaginal candidiasis it was observed that once animals cleared the infection a second challenge resulted in an enhanced delayed hypersensitivity, reduced numbers of Candida in the vagina and less hyphal penetration of the mucosa [156]. Furthermore, the protective effects of the primary infection did not protect mice from gastrointestinal or systemic candidiasis [156]. These studies present the interesting possibility that vaccines should be site directed.

A desirable aspect for using a vaccine would be one that would remain effective in immunocompromised patients or could be used in immunocompromised patients [373]. In murine studies of aspergillosis, Ito et al. [374] demonstrated vaccine efficacy in corticosteroid suppressed animals. Although CD4+ cells are a primary cell involved in protective immunity resulting from vaccines, more recent studies have demonstrated that CD8+ cells also have a role to play. Long-lived memory CD8+ cells could induce and mediate resistance to H. capsulatum and B. dermatitidis in absence of CD4+ subsets [375], as well as Coccidioides [376]. These findings are especially encouraging since potentiation of CD8+ cells could be designed to protect even immunosuppressed patients.

**Antifungal therapy**

The ever increasing numbers of fungal infections, whether primary or opportunistic, especially over the last two decades, has proven again and again the limitations of the antifungal armamentarium. Although the drugs have demonstrated valuable activity against many mycoses, therapy is not always effective. Clinical experience tells us that additional studies are needed in order to find more effective drugs, new routes of administration or new regimens, and animal models play an important role in refining monotherapy with antifungal drugs. Each class of drugs has limitations in spectrum, cidal activity and utility as a result of pharmacodynamics. In addition, the increasing occurrence of unusual fungi as opportunistic pathogens, such as the Zygomycetes or other filamentous fungi such as Fusarium, which are innately resistant to almost all antifungal drugs, places the development of new and effective therapies as a prime pursuit [377,378].

In therapeutics, the gold standard is the (double-blind) randomized clinical trial. However, when performing clinical trials one has to know the right question to address before embarking on a big adventure. Moreover, such clinical trials usually require many patients, are expensive, sometimes must compete with other studies for the same patients, usually require coordination of multiple centers, take a long time, and are confounded by variations in treatment groups (different stages of disease when therapy starts, co-morbidities not randomly distributed, different ages, sex, genetic backgrounds).

When a randomized clinical trial is not possible, the alternatives are clinical case series and open trials, and the deficiencies are that the plural of anecdote is not data. Animal model studies can tell you which clinical trials you should try to do, can be fashioned to mimic a variety of human diseases, are relatively inexpensive, provide sufficient subjects for appropriate statistical analysis, can be relatively easily repeated (at which time study conditions can be intentionally varied), and give prompt answers (enabling decisions about future directions). All the subjects can be the same age and sex, be genetically identical, and have the same co-morbidity (or none) and same stage of disease at entry. Different regimens can be studied concurrently to avoid unknown external factors (e.g., referral patterns, treatments for co-morbidities) influencing outcome that are changing over time, as occurs in the clinical setting. The outcome endpoints can be devoid of subjectivity (not ‘the patient is improved/stable/worse’). While death can be an objective endpoint in clinical trials or animal models, in models the amount of infection after therapy can be quantified, even in survivors. One can do pharmacologic and toxicologic studies not possible in patients, including sampling parts of or whole organs for histopathology and/or drug concentration, and study uncommon conditions that would be difficult or impossible to study in a randomized clinical trial, because of insufficient numbers of subjects available in a reasonable time frame.
Animal models have been used extensively to test experimental antifungal therapy against localized or systemic infections by clinical important fungi, and past experience demonstrates that efficacy in one or more animal models is predictive of efficacy clinically. Perhaps no other use of animal models can be so directly and promptly linked to benefits in the care of patients. Studies in animals are directed to test the efficacy and safety of new agents, as well as new indications for licensed molecules. After in vitro tests have shown efficacy against a fungus, animal modeling is a mandatory step prior to clinical trials and final governmental approval. Once a model of infection has been established, demonstrating reproducibility and mimicking the clinical disease, determination of the route and regimen of administration, pharmacokinetic/pharmacodynamics, efficacy and toxicity of the drug may be tested. Varying the time of onset of therapy in animal models can give valuable clues as to the importance of timing of initiation of therapy in clinical diseases. The types of studies performed are those of monotherapy, combination therapy and pharmacokinetic or pharmacodynamics studies. We, and others, have reviewed these types of studies extensively and will thus present only examples of the types of studies done [35,36,38,41,42,107,108,312,379–382].

### Monotherapy studies

Determination of drug efficacy in an animal model is assessed by many investigators using the parameters of survival and fungal burdens in the tissues, by colony-forming units, after a defined period of dosing. Several aspects of performing these types of studies need to be considered. Many of these already have been discussed in the initial sections of this review. Based on in vitro susceptibility studies it is critical to know which organisms within a genus appear to be susceptible and which are resistant to the antifungal being tested, as well as how the organisms behave during the experimental infection. Similarly, one must be aware of differences between the animal species being used for the models, as the efficacy of antifungal drug can be also species-dependent. Because of physiological differences between animal species, the pharmacokinetic and pharmacodynamics of the new drug must be considered before drug testing in vivo. The example of VCZ in guinea pigs versus mice has been discussed in a previous section of this review. However, administration of grapefruit juice in the drinking water of mice, which inhibits gut P450 enzymes, has been shown to increase the serum concentration of the drug to therapeutic levels [383,384] and allows the use of murine models to test VCZ efficacy against different experimental infections [98,101,385]. Variability in drug efficacy can also depend on the animal model used and for this reason data should be carefully interpreted, as treatment efficacy in one model may not occur in a different one. For example, we have previously reported differences in efficacy for several antifungal drugs between murine models of systemic and pulmonary aspergillosis [386,387].

A true benefit of using animal models for the determination of drug efficacy is that doses and regimens can be easily monitored, and modified in animal models, in order to establish an optimal effect [36,37,388]. Higher dosages of an antifungal are not always more curative, and the use of animal models has clearly demonstrated this for the lipid-carried amphotericin B preparations and the echinocandins in murine models of CNS aspergillosis [2,5,385,387,389].

As noted previously, animal models are used in lieu of clinical trials where those trials may be difficult to perform or put patients at serious risk. A more recent example of the utility of a model is that of a rabbit model of indwelling catheter colonization by a biofilm of *C. albicans*, which mimics the very serious clinical problem of candidal line infections and sepsis. These studies demonstrated the effectiveness of using liposomal amphotericin B to clear the biofilm from the catheter [390] and also demonstrated ethanol inhibition of biofilm formation by *C. albicans in vivo*, which may suggest novel methods of clearing the catheter [391]. Studies such as this directly address important clinical problems and can be indicative of better methods of treatment.

### Combination therapy studies

Another strategy in face of serious fungal disease is the use of combinations of drugs. Combination therapy can enhance efficacy over monotherapy, avoid toxic effects of one of both drugs by reducing the dosage and increase the therapeutic benefits against infections caused by multi-resistant species. When performing antifungal combination studies it is critical to know the efficacy of the monotherapies being considered for use in combination. Significant enhancement of efficacy by the combination cannot be demonstrated if one or both of the compounds is already curative at the dosage being tested. Thus, if one or both drugs alone show good efficacy, the combination of the two is assessed by using them at suboptimal dosages [36,37]. The combined efficacy over the monotherapy must be seen in animal models by comparing the combination and demonstrating a significant enhancement in efficacy over that of either drug tested alone at the same dosing...
concentration. Combination can be done by administering both drugs simultaneously or sequentially.

Recently published examples of combination therapy studies demonstrated that lipid-carried AMB in combination with VCZ given concurrently or sequentially with AMB for three days followed by VCZ showed greater efficacy against CNS aspergillosis than did the respective monotherapies [385,387,389]. In another study, liposomal AMB followed by administration of VCZ was superior to VCZ followed by L-AMB [91].

A variety of drugs have been studied in combination particularly in murine models of candidiasis and aspergillosis. The combinations include amphotericin B preparations with azoles or echinocandins and azoles and echinocandins. Of note and discussed earlier in this review are the use of immunostimulatory cytokines in combination with a conventional antifungal agent such as amphotericin B or fluconazole. IL-12, and IFN-γ particularly, have been shown to be useful adjuncts to conventional therapy in a number of murine models including cryptococcosis and histoplasmosis [107,213,228,229,311,312,324,392].

Pharmacokinetic and pharmacodynamic studies

Determination of in vitro pharmacological parameters, such as $C_{\text{max}}$, $t_{1/2}$, $\text{AUC}_{0-24}$, volume of distribution and elimination and toxicity, is necessary for antifungal drugs as well. Numerous studies of this type have used mice and rabbits and are usually performed in uninfected animals [102,393–395]. Less frequent and sorely needed are pharmacodynamic studies where the same pharmacokinetic parameters are determined in infected animals [102,394,395]. Important for the future is the application of physiologically based pharmacokinetics to antifungal agents. These types of studies are done modeling pharmacokinetic parameters for two or more animal species in order to predict the parameters in humans. Studies of this type are very limited with antifungal drugs and one example is a study with terbinafine done in rats showing the modeling from rat data correlated well with published human data [396]. Additional studies on other antifungal drugs are much needed.

A subdivision of pharmacologic studies is toxicologic studies. As mentioned in regard to other pharmacologic studies, these are usually performed in uninfected animals, often the same species in which an efficacy trial and/or a pharmacologic study are conducted. Early studies of polyene toxicity to nephrons, and the localization of the effect, necessarily relied on animal models. As examples of more recent studies, we have utilized animal models to illuminate endocrinological effects of azoles [397], drug-drug interactions resulting in toxicity [398] and to study the neurotoxicity of amphotericin preparations [399], information that would not have been possible to obtain from humans. Toxicological studies in infected animals are also sorely needed, largely because of the possibility that disease toxicity and drug toxicity may summate, such as that reported in studies of murine blastomycosis [400].

Developing diagnostic tools

Surrogate markers of infection and alternatives to survival or infectious burden assays have included radiographic imaging techniques applicable to pulmonary models of aspergillosis in rabbits [3,35,65,81], metabolites [401] and other clinical parameters such as body weight and temperature. In addition to the PCR methods, assays for antigenemia (galactomannan and glucan) have been used to study disease progression, as well as for diagnostic purposes [401–406]. Rabbit studies with aspergillosis have shown that galactomannan assays are useful for diagnostics and gave results similar to those from humans [3,402,404,407,408]. The course of cryptococcal polysaccharide concentrations in animal serum in the presence or absence of therapy corroborates correlations of the concentrations, fungal load, and disease severity in humans [111,409,410]. In each example mentioned, the clinical utility of a particular assay is based on the corroboration of animal data in later clinical experience.

Summary and conclusions

A primary aim of this review has been to demonstrate to the reader that animal models in mycology are a useful and necessary tool for studies of pathogenesis, host-response, clinical infection and therapeutics. We have discussed various aspects of the benefits and weaknesses, types of data generated and evaluation of these data. From the numerous fungal animal models discussed it should be apparent that each is unique and requires careful performance and evaluation before reasonable conclusions concerning the results can be drawn. One cannot make overall generalizations that all fungal infections progress or behave in the same manner nor does the host respond in the same way to all fungi. Thus, before embarking on studies utilizing animals a series of questions must be addressed and answered by the investigator. What is the infection being modeled and is the model well defined and reproducible? What is the immune status of the host? Is the fungal infection being modeled an acute or chronic disease? When therapeutic studies are the goal, does the investigator...
know what the efficacies of monotherapy regimens are, what are the optimal doses, duration, frequency and routes of therapy? Regardless of the model and purpose of using the model, what parameters of infection should be followed? Are these parameters survival and CFU or is a surrogate marker of infection applicable? And finally, how are the data best evaluated, with which statistical analyses? Answering these questions first will lead to better experimental results, sound conclusions and allow us to better understand medically important fungi and their interactions with the host.

References

1 Spellberg B, Ibrahim AS, Edwards JE, Jr., Filler SG. Mice with disseminated candidiasis die of progressive sepsis. J Infect Dis 2005; 192: 336–343.
2 Imai J, Singh G, Fernandez B, Clemons KV, Stevens DA. Efficacy of Abelcet and caspofungin, alone or in combination, against aspergillosis in a murine model. J Antimicrob Chemother 2005; 56: 166–171.
3 Latge JP. Aspergillus fumigatus and aspergillosis. Clin Microbiol Rev 1999; 12: 310–350.
4 Bowman JC, Abruzzo GK, Anderson JW, et al. Quantitative PCR assay to measure Aspergillus fumigatus burden in a murine model of disseminated aspergillosis: demonstration of efficacy of caspofungin acetate. Antimicrob Agents Chemother 2001; 45: 3474–3481.
5 Singh G, Imai J, Clemons KV, Stevens DA. Efficacy of caspofungin against central nervous system Aspergillus fumigatus infection in mice determined by TaqMan PCR and CFU methods. Antimicrob Agents Chemother 2005; 49: 1369–1376.
6 Balloy V, Huerre M, Latge JP, Chignard M. Differences in patterns of infection and inflammation for corticosteroid treatment and chemotherapy in experimental invasive pulmonary aspergillosis. Infect Immun 2005; 73: 494–503.
7 U.S. Congress Office of Technology Assessment. Alternatives to animal use in research, testing and education. Washington, DC: Government Printing Office, 1986.
8 Tournu H, Serneels J, Van Dijck P. Fungal pathogens research: novel and improved molecular approaches for the discovery of antifungal drug targets. Curr Drug Targets 2005; 6: 909–922.
9 Fuchs BB, Mylonakis E. Using non–mammalian hosts to study fungal virulence and host defense. Curr Opin Microbiol 2006; 9: 346–351.
10 Alarco AM, Marcil A, Chen J, et al. Immune-deficient Drosophila melanogaster: a model for the innate immune response to human fungal pathogens. J Immunol 2004; 172: 5622–5628.
11 Chamilos G, Lionakis MS, Lewis RE, et al. Drosophila melanogaster as a facile model for large-scale studies of virulence mechanisms and antifungal drug efficacy in Candida species. J Infect Dis 2006; 193: 1014–1022.
12 Stroschein-Stevenson SL, Foley E, O’Farrell PH, Johnson AD. Identification of Drosophila gene products required for phagocytosis of Candida albicans. PLoS Biol [serial on the internet] Jan. 2006; 4(1): [about 4 pp]. Available from: http://biology.plosjournals.org/perlserv/?request=get-document&doi=10.1371/journal.pbio.0040004&ct=1
13 Steenbergen JN, Shuman HA, Casademav A. Cryptococcus neoformans interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. Proc Natl Acad Sci USA 2001; 98: 15245–15250.
14 Bunting LA, Neilson JB, Bulmer GS. Cryptococcus neoformans: a gastronomic delight of a soil ameba. Sabouraudia 1979; 17: 225–232.
15 Land KM. Soil amoebae help to unravel fungal pathogenesis. Trends Microbiol 2002; 10: 116.
16 Greub G, Raout D. Microorganisms resistant to free-living amoebae. Clin Microbiol Rev 2004; 17: 413–433.
17 Steenbergen JN, Nosanchuk JD, Malliaris SD, Casademav A. Interaction of Blastomyces dermatitidis, Sporothrix schenckii, and Histoplasma capsulatum with Acanthamoeba castellanii. Infect Immun 2004; 72: 3478–3488.
18 Malliaris SD, Steenbergen JN, Casademav A. Cryptococcus neoformans var. gattii can exploit Acanthamoeba castellanii for growth. Med Mycol 2004; 42: 149–158.
19 Steenbergen JN, Nosanchuk JD, Malliaris SD, Casademav A. Cryptococcus neoformans virulence is enhanced after growth in the genetically malleable host Dictyostelium discoideum. Infect Immun 2003; 71: 4862–4872.
20 Steinert M, Heuner K. Dictyostelium as host model for pathogenesis. Cell Microbiol 2005; 7: 307–314.
21 Frases S, Chaskes S, Dadachova E, Casademav A. Induction by Klebsiella aerogenes of a melanin-like pigment in Cryptococcus neoformans. Appl Environ Microbiol 2006; 72: 1542–1550.
22 Unal C, Steinert M. Dictyostelium discoideum as a model to study host-pathogen interactions. Methods Mol Biol 2006; 346: 507–515.
23 Mylonakis E, Aballay A. Worms and flies as genetically tractable animal models to study host-pathogen interactions. Infect Immun 2005; 73: 3833–3841.
24 Balloy V, Si-Tahar M, Takeuchi O, et al. Involvement of toll-like receptor 2 in experimental invasive pulmonary aspergillosis. Infect Immun 2005; 73: 5420–5425.
25 Lionakis MS, Kontoyiannis DP. Fruit flies as a minihost model for studying drug activity and virulence in Aspergillus. Med Mycol 2005; 43(Suppl. 1): S111–114.
26 Netea MG, Van der Graaf C, Van der Meer JW, Kullberg BJ. Recognition of fungal pathogens by Toll-like receptors. Eur J Clin Microbiol Infect Dis 2004; 23: 672–676.
27 Rooder A, Kirschning CJ, Rupe RA, Schaller M, Korting HC. Toll-like receptors and innate antifungal responses. Trends Microbiol 2004; 12: 44–49.
28 Renwick J, Daly P, Reeves EP, Kavanagh K. Susceptibility of larvae of Galleria mellonella to infection by Aspergillus fumigatus is dependent upon stage of conidial germination. Mycopathologia 2006; 161: 377–384.
29 Reeves EP, Reiber K, Neville C, et al. A nonbrowsable peptide synthetase (Pse1) confers protection against oxidative stress in Aspergillus fumigatus. FEBS J 2006; 273: 3038–3053.
30 Scully LR, Bidochka MJ. Serial passage of the opportunistic pathogen Aspergillus flavus through an insect host yields decreased saprobic capacity. Can J Microbiol 2005; 51: 185–189.
31 Reeves EP, Messina CG, Doyle S, Kavanagh K. Correlation between gliotoxin production and virulence of Aspergillus fumigatus in Galleria mellonella. Mycopathologia 2004; 158: 73–79.
32 St Leger RJ, Screen SE, Shams-Pirzadeh B. Lack of host specialization in Aspergillus flavus. Appl Environ Microbiol 2000; 66: 320–324.
33 Bhabra R, Miley MD, Mylonakis E, et al. Disruption of the Aspergillus fumigatus gene encoding nucleolar protein CgrA.
impaired thermotolerant growth and reduces virulence. Infect Immun 2004; 72: 4731–4740.

34 Sorensen KN, Clemons KV, Stevens DA. Murine models of blastomycosis, coccidiodomycosis, and histoplasmosis. Mycopathologia 1999; 146: 53–65.

35 Clemons KV, Stevens DA. The contribution of animal models of aspergillosis to understanding pathogenesis, therapy and virulence. Med Mycol 2005; 43(Suppl. 1): S101–S110.

36 Clemons KV, Stevens DA. Animal models testing monotherapy versus combination antifungal therapy: lessons learned and future directions. Curr Opin Infect Dis 2006; 19: 360–364.

37 Clemons KV, Stevens DA. Animal models of Aspergillus infection in preclinical trials, diagnostics and pharmacodynamics: What can we learn from them? Med Mycol 2006; 44(Suppl. 1): S119–S126.

38 Polak A. Experimental models in antifungal chemotherapy. Mycoses 1998; 41: 1–30.

39 Brummer E, Clemons KV. Animal models in systemic mycoses. In: Miyaji M (ed.). Animal Models in Medical Mycology. Boca Raton, FL: CRC Press, 1987: 79–100.

40 de Repentigny L. Animal models in the analysis of Candida-host-pathogen interactions. Curr Opin Microbiol 2004; 7: 324–329.

41 Stevens DA. Animal models of blastomycosis. Semin Respir Infect 1997; 12: 196–197.

42 Stevens DA. Animal models in the evaluation of antifungal drugs. J Mycol Medica 1996; 6: 7–10.

43 Stevens DA. Animal models to evaluate antifungal activity: utility of models in chronic deep mycoses. In: Iwata K, Vanden Bossche H (eds). In Vitro and In Vivo Evaluation of Antifungal Agents. Amsterdam, NY: Elsevier Science, 1986: 153–157.

44 Stewart NJ, Munday BL. Possible differences in pathogenicity of Histoplasma capsulatum in the rabbit. J Med Vet Mycol 1986; 24: 369–376.

45 Ishibashi Y, Kaufman HE, Matsumoto T, Kagawa S. The contribution of animal models of C. albicans to understanding pathogenesis, therapy and virulence. Jpn J Ophthalmol 1999; 43: 127–132.

46 Castanon-Olivares LR, Manzano-Gayoso P, Lopez-Martinez R, De la Rosa-Velazquez IA, Soto-Reyes-Solis E. Effectiveness of terbinafine in the eradication of Microsporum canis from laboratory cats. Mycoses 2001; 44: 95–97.

47 Tesh RB, Schneidau JD, Jr. Experimental infection of North American insectivorous bats (Artibeus lituratus) with H. capsulatum. J Infect Dis 1981; 143: 550–554.

48 Waterston RH, Lindblad-Toh K, Birney E, et al. Initial sequencing and comparative analysis of the mouse genome. Nature 2002; 420: 520–562.

49 Williams PL, Sobel RA, Sorensen KN, et al. A model of coccidioidal meningoencephalitis and cerebrospinal vasculitis in the rabbit. J Infect Dis 1998; 178: 1217–1221.

50 Perfect JR, Lang SD, Durack DT. Chronic cryptococcal meningitis: a new experimental model in rabbits. Am J Pathol 1980; 101: 177–194.

51 Jafari HS, Saez-Llorens X, Grimprel E, et al. Characteristics of experimental Candida albicans infection of the central nervous system in rabbits. J Infect Dis 1991; 164: 389–395.

52 Singh SM, Sharma S, Chatterjee PK. Clinical and experimental mycotic keratitis caused by Aspergillus terreus and the effect of subconjunctival oxiconazole treatment in the animal model. Mycopathologia 1990; 112: 127–137.

53 Demant E, Easterbrook M. An experimental model of Candida endophthalmitis. Can J Ophthalmol 1977; 12: 304–307.

54 Goldblum D, Frueh BE, Sarra GM, Katsoulis K, Zimmerli S. Topical caspofungin for treatment of keratitis caused by Candida albicans in a rabbit model. Antimicrob Agents Chemother 2005; 49: 1359–1363.

55 Isobe Y, Hatano H. Study of suppressive effect of intravenous fluconazole on endogenous Candida endophthalmitis in rabbits. Jpn J Ophthalmol 1992; 36: 23–27.

56 O’Day DM, Head WS, Robinson RD, Williams TE, Wolf R. Ocular pharmacokinetics of saperconazole in rabbits. A potential agent against keratomycoses. Arch Ophthalmol 1992; 110: 550–554.

57 Fujita NK, Henderson DK, Hockey LJ, Guze LB, Edwards JE, Jr. Comparative ocular pathogenicity of Cryptococcus neoformans, Candida glabrata, and Aspergillus fumigatus in the rabbit. Invest Ophthalmol Vis Sci 1982; 22: 410–416.

58 Smith RE, O’Connor GR, Halde CJ, Scalaret MEA, Easterbrook WM. Clinical course in rabbits after experimental inoculation of ocular histoplasmosis. Am J Ophthalmol 1973; 76: 284–293.

59 Ishibashi Y, Kaufman HE, Matsumoto T, Kagawa S. The contribution of animal models of C. albicans to understanding pathogenesis, therapy and virulence. Jpn J Ophthalmol 1999; 43: 127–132.

60 Ishibashi Y, Kaufman HE, Matsumoto T, Kagawa S. The contribution of animal models of C. albicans to understanding pathogenesis, therapy and virulence. Jpn J Ophthalmol 1999; 43: 127–132.

61 McGuire TW, Bullock JD Jr., Bullock JD, Elder BL, Finkhouser JW. Fungal endophthalmitis. An experimental study with a review of 17 human ocular cases. Arch Ophthalmol 1991; 109: 1289–1296.

62 Letterio JJ, Lehrrecher B, Pollack G, Walsh TJ, Chonanck SJ. Invasive candidiasis stimulates hepatocyte and monocyte production of active transforming growth factor beta. Infect Immun 2001; 69: 5155–5120.

63 Patterson TF, Miniter P, Dijkstra J, et al. Treatment of experimental invasive aspergillosis with novel amphotericin B/cholesterol-sulfate complexes. J Infect Dis 1989; 157: 171–724.

64 Patterson TF, Miniter P, Ryan JL, Andriole VT. Effect of immunosuppression and amphotericin B on Aspergillus antigenemia in an experimental model. J Infect Dis 1988; 158: 415–422.

65 Walsh TJ, Garrett K, Feurerstein E, et al. Therapeutic monitoring of experimental invasive pulmonary aspergillosis by ultrafast computerized tomography, a novel, noninvasive method for measuring responses to antifungal therapy. Antimicrob Agents Chemother 1995; 39: 1065–1069.

66 Walsh TJ, Petraitis V, Petraitiene R, et al. Experimental pulmonary aspergillosis due to Aspergillus terreus: pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. J Infect Dis 2003; 188: 305–319.

67 Capilla J, Yustes C, Mayayo E, et al. Efficacy of albaconazole (UR-9825) in treatment of disseminated Scedosporium prolificans in treatment of disseminated Scedosporium prolificans infection in rabbits. Antimicrob Agents Chemother 2003; 47: 1948–1951.

68 Chilvers ER, Spreadbury CL, Cohen J. Bronchoalveolar lavage in an immunosuppressed rabbit model of invasive pulmonary aspergillosis. Mycopathologia 1989; 108: 163–171.

69 Groll AH, Gonzalez CE, Giri N, et al. Liposomal nystatin against experimental pulmonary aspergillosis in persistently neutropenic rabbits: efficacy, safety and non-compartmental pharmacokinetics. J Antimicrob Chemother 1999; 43: 95–103.

70 Nakamura T. Experimental pulmonary candidiasis in modified rabbits. Histopathological, ultrastructural and enzyme cyto-
chemical studies of tissue reactions. Mycopathologia 1984; 85: 129–144.

71 Nakamura T. Experimental pulmonary candidiasis in modified rabbits. II. Immunohistochemical evidence of participation of immune complexes in the formation of fungal lesions in C. albicans-sensitized hosts. Mycopathologia 1985; 92: 93–99.

72 Gross NT, Jarstrand C, Robertson B. Treatment of experimental Cryptococcus neoformans infection in newborn rabbits by airway instillation of specific antibody and surfactant. Lett Appl Microbiol 2000; 31: 218–222.

73 Chakrabarti A, Jatana M, Sharma SC. Rabbit as an animal model of parasinal sinus mycoses. J Med Vet Mycol 1997; 35: 295–297.

74 Dufour X, Kauffmann-Lacroix C, Goujon JM, et al. Experimental model of fungal sinusitis: a pilot study in rabbits. Ann Otol Rhinol Laryngol 2005; 114: 167–172.

75 Kamei K. Animal models of zygomycosis. In vitro antifungal activity of ketoconazole, zinc pyrithione, and selenium sulfide against Pityrosporum ovale in guinea pigs. J Am Acad Dermatol 2002; 46: 2564–2568.

76 Carrizosa J, Kohn C, Levison ME. Experimental aspergillus endocarditis in rabbits. J Lab Clin Med 1975; 86: 746–753.

77 Demierre G, Freedman LR. Experimental endocarditis: prophylaxis of Candida albicans infections by 5-fluorocytosine in rabbits. Antimicrob Agents Chemother 1979; 16: 252–254.

78 Padula A, Chambers HF. Evaluation of ciclofungin (LY121019) for treatment of experimental Candida albicans endocarditis in rabbits. Antimicrob Agents Chemother 1989; 33: 1822–1823.

79 Kurup VP, Gruing N. Animal models of allergic bronchopulmonary aspergillosis. Mycopathologia 2002; 153: 165–177.

80 Kurup VP, Sheth NK. Experimental aspergillosis in rabbits. Comp Immunol Microbiol Infect Dis 1981; 4: 161–174.

81 Patterson TF. The future of animal models of invasive aspergillosis. J Antimicrob Chemother 2002; 49: 353–357.
109 Morozumi PA, Brummer E, Stevens DA. Strain differences in resistance to infection reversed by route of challenge: studies in blastomycosis. Infect Immun 1981; 34: 623-625.

110 Curtis JL, Huffnagle GB, Chen GH, et al. Experimental murine pulmonary cryptococcosis. Differences in pulmonary inflammation and lymphocyte recruitment induced by two encapsulated strains of Cryptococcus neoformans. Lab Invest 1994; 71: 113-126.

111 Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS – 100 years after the discovery of Cryptococcus neoformans. Clin Microbiol Rev 1993; 8: 515-548.

112 Ortoneda M, Pastor FJ, Mayayo E, Guarrro J. Comparison of the experimental infection with Scedosporium prolificans in different origins in a murine model. J Med Microbiol 2002; 51: 924-928.

113 Cano J, Guaro J, Mayayo E, Fernandez-Ballart J. Experimental infection with Scedosporium inflatum. J Med Vet Mycol 1992; 30: 413-420.

114 Kan VL, Bennett JE. Efficacies of four antifungal agents in experimental murine sporotrichosis. Antimicrob Agents Chemother 1988; 32: 1619-1623.

115 Brummer E, Castaneda E, Restrepo A. Paracoccidioidomycosis: an update. Clin Microbiol Rev 1993; 6: 89-117.

116 Kudeken N, Kawakami K, Kusano N, Saito A. Cell-mediated immunity in host resistance against infection caused by Pencillium marneffei. J Med Vet Mycol 1996; 34: 371-378.

117 Carmen-Vivas JR, Torres-Rodriguez JM, Corominas JM, Madrenys N. Pathogenicity of Hansenula anomala in a model of immunocompromised mice. Mycopathologia 1998; 144: 67-71.

118 Mayayo E, Pujol I, Guaro J. Experimental pathogenicity of four opportunistic Fusarium species in a murine model. J Med Microbiol 1999; 48: 363-366.

119 Chiller TM, Luque JC, Sobel RA, et al. Development of a murine model of cerebral aspergillosis. J Infect Dis 2002; 186: 574-577.

120 Blasi E, Barluzzi R, Mazzolla R, Mosci P, Bistoni F. Experimental model of intracerebral infection with Cryptococcus neoformans: roles of phagocytes and opsonization. Infect Immun 1992; 60: 3682-3688.

121 Chiller TM, Sobel RA, Luque JC, Clemons KV, Stevens DA. Efficacy of amphotericin B or itraconazole in a murine model of central nervous system Aspergillus infection. Antimicrob Agents Chemother 2003; 47: 813-815.

122 Capilla J, Mayayo E, Serena C, Pastor FJ, Guaro J. A novel murine model of cerebral scedosporiosis: lack of efficacy of amphotericin B. J Antimicrob Chemother 2004; 54: 1092-1095.

123 Kleinschmidt-DeMasters BK. Central nervous system aspergillosis: a 20-year retrospective series. Hum Pathol 2002; 33: 116-124.

124 Allendoerfer R, Yates RR, Marquis AJ, et al. Comparison of SCH 39304 and its isomers, RR 42427 and SS 42426, for treatment of murine cryptococciosis and coccidioidal meningitis. Antimicrob Agents Chemother 1992; 36: 217-219.

125 Graybill JR, Sun SH, Ahrens J. Treatment of murine coccidioidal meningitis with fluconazole (UK 49,858). J Med Vet Mycol 1986; 24: 113-119.

126 Haynes RR, Connolly PA, Durkin MM, et al. Antifungal therapy for central nervous system histoplasmosis, using a newly developed intracranial model of infection. J Infect Dis 2002; 185: 1830-1832.

127 Hossain MA, Mukherjee PK, Reyes G, Long L, Ghannoum MA. Effects of fluconazole singly and in combination with 5-fluorocytosine or amphotericin B in the treatment of cryptococcal meningoencephalitis in an intracranial murine model. J Chemother 2002; 14: 351-360.

128 Craven PC, Graybill JR. Combination of oral flucytosine and ketoconazole as therapy for experimental cryptococcal meningitis. J Infect Dis 1984; 149: 584-590.

129 Kamberi P, Sobel RA, Clemons KV, et al. A murine model of coccidioidal meningitis. J Infect Dis 2003; 187: 453-460.

130 Harvey RP, Schmid ES, Carrington CC, Stevens DA. Mouse model of pulmonary blastomycosis: utility, simplicity, and quantitative parameters. Am Rev Respir Dis 1978; 117: 695-703.

131 Lima C, Vital JP. Olfactory mucosa response in guinea pigs following intranasal instillation with Cryptococcus neoformans. A histological and immunocytochemical study. Mycopathologia 1994; 126: 65-73.

132 Waldorf AR, Halde C, Vedros NA. Murine model of pulmonary mucormycosis in cortisone-treated mice. Sabouraudia 1982; 20: 217-224.

133 Osier M, Oberdorster G. Intratracheal inhalation vs intratracheal instillation: differences in particle effects. Fundam Appl Toxicol 1997; 40: 220-227.

134 Sheppard DC, Graybill JR, Najvar LK, et al. Standardization of an experimental murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 2006; 50: 3501-3503.

135 Sheppard DC, Rieg G, Chiang LY, et al. Novel inhalational murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 2004; 48: 1908-1911.

136 Cole GT, Halawa AA, Anaissie EJ. The role of the gastrointestinal tract in hematogenous candidiasis: from the laboratory to the bedside. Clin Infect Dis 1996; 22(Suppl. 2): S73-88.

137 Samaranayake YH, Samaranayake LP. Experimental oral candidiasis in animal models. Clin Microbiol Rev 2001; 14: 398-429.

138 Cole GT, Lynn KT, Seshan KR. An animal model for oropharyngeal, esophageal and gastric candidosis. Mycososes 1990; 33: 7-19.

139 Clemons KV, Gonzalez GM, Singh G, et al. Development of an orogastrointestinal mucosal model of candidiasis with dissemination to visceral organs. Antimicrob Agents Chemother 2006; 50: 2650-2657.

140 Samonis G, Karyotakis NC, Anaissie EJ, et al. Effects of cyclophosphamide and ceftriaxone on gastrointestinal colonization of mice by Candida albicans. Antimicrob Agents Chemother 1996; 40: 2221-2223.

141 Sandovsky-Losika H, Barr-Nea L, Segal E. Fatal systemic candidiasis of gastrointestinal origin: an experimental model in mice compromised by anti-cancer treatment. J Med Vet Mycol 1992; 30: 219-231.

142 Sandovsky-Losika H, Segal E. Interaction of Candida albicans with murine gastrointestinal mucosa: effect of irradiation on adherence in vitro. J Med Vet Mycol 1989; 27: 345-352.

143 Sandovsky-Losika H, Segal E. Interaction of Candida albicans with murine gastrointestinal mucosa from methotrexate and 5-fluorouracil treated animals: in vitro adhesion and prevention. J Med Vet Mycol 1990; 28: 279-287.

144 Clemons KV, Stevens DA. Treatment of orogastrointestinal candidiasis in SCID mice with fluconazole alone or in combination with recombinant granulocyte colony-stimulating factor or interferon-gamma. Med Microol 2000; 38: 213-219.

145 Narayanan R, Joyce WA, Greenfield RA. Gastrointestinal candidiasis in a murine model of severe combined immunodeficiency syndrome. Infect Immun 1991; 59: 2116-2119.

146 Fidel PL, Jr., Sobel JD. Immunopathogenesis of recurrent vulvovaginal candidiasis. Clin Microbiol Rev 1996; 9: 335-348.
147 Sobel JD, Muller G, McCormick JF. Experimental chronic vaginal candidosis in rats. Sabouraudia 1985; 23: 199–206.

148 Steele C, Ratterree M, Fidel PL, Jr. Differential susceptibility of two species of macaques to experimental vaginal candidiasis. J Infect Dis 1999; 180: 802–810.

149 Fidel PL, Jr.,Cutright J, Steele C. Effects of reproductive hormones on experimental vaginal candidiasis. Infect Immun 2000; 68: 651–657.

150 Spearow JL, O’Henley P, Doemeny P, et al. Genetic variation in physiological sensitivity to estrogen in mice. APMS 2001; 109: 356–364.

151 Calderon L, Williams R, Martinez M, Clemons KV, Stevens DA. Genetic susceptibility to vaginal candidiasis. Med Mycol 2003; 41: 143–147.

152 Clemens KV, Spearow JL, Parmar R, Espiritu M, Stevens DA. Genetic susceptibility of mice to *Candida albicans* vaginitis correlates with host estrogen sensitivity. Infect Immun 2004; 72: 4878–4880.

153 De Bernardis F, Cassone A, Sturtevant J, Calderone R. Expression of *Candida albicans* SAP1 and SAP2 in experimental vaginitis. Infect Immun 1995; 63: 1887–1892.

154 de Bernardis F, Morelli L, Ceddia T, Lorenzini R, Cassone A. Experimental pathogenicity and acid proteinase secretion of vaginal isolates of *Candida parapsilosis*. J Med Vet Mycol 1990; 28: 125–137.

155 Fidel PL, Jr.,Cutright JL, Tait L, Sobel JD. A murine model of *Candida glabrata* vaginitis. J Infect Dis 1996; 173: 425–431.

156 Fidel PL, Jr., Lynch ME, Conaway DH, Tait L, Sobel JD. Mice immunized by primary vaginal *Candida albicans* infection develop acquired vaginal mucosal immunity. Infect Immun 1995; 63: 547–553.

157 Fidel PL, Jr., Lynch ME, Sobel JD. *Candida*-specific cell-mediated immunity is demonstrable in mice with experimental vaginal candidiasis. Infect Immun 1993; 61: 1990–1995.

158 Fidel PL, Jr., Sobel JD. Protective immunity in experimental *Candida* vaginitis. Res Immunol 1998; 149: 361–373.

159 MacCallum DM, Odds FC. Temporal events in the intravaginal challenge model for experimental *Candida albicans* infections in female mice. Mycoses 2005; 48: 151–161.

160 Baghian A, Lee KW. Systemic candidosis in beige mice. J Med Vet Mycol 1989; 27: 51–55.

161 Capilla J, Maftei CM, Clemens KV, Sobel RA, Stevens DA. Experimental systemic infection with *Cryptococcus neoformans var. grubii* and *Cryptococcus gattii* in normal and immunodeficient mice. Med Mycol 2006; 44: 601–610.

162 Kwon-Chung KJ, Rhodes JC. Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. Infect Immun 1986; 51: 218–223.

163 Jacobson ES. Pathogenic roles for fungal melanins. Clin Microbiol Rev 2000; 13: 708–717.

164 Nosanchuk JD, Casadevall A. The contribution of melanin to microbial pathogenesis. Cell Microbiol 2003; 5: 203–223.

165 Kwon-Chung KJ, Polacheck I, Popkin TJ. Melanin-lacking mutants of *Cryptococcus neoformans* and their virulence for mice. J Bacteriol 1982; 150: 1414–1421.

166 Dixon DM, Polak A, Szaniszlo PJ. Pathogenicity and virulence of wild-type and melanin-deficient *Wangiella dermatitidis*. J Med Vet Mycol 1987; 25: 97–106.

167 Dixon DM, Migliozzi J, Cooper CR, Jr., et al. Melanized and non-melanized multicellular form mutants of *Wangiella dermatitidis* in mice: mortality and histopathology studies. Mycoses 1992; 35: 17–21.

168 Jahn B, Koch A, Schmidt A, et al. Isolation and characterization of a pigmentless-conidium mutant of *Aspergillus fumigatus* with altered conidial surface and reduced virulence. Infect Immun 1997; 65: 5110–5117.

169 Tsai HF, Chang YC, Washburn RG, Wheeler MH, Kwon-Chung KJ. The developmentally regulated *alb1* gene of *Aspergillus fumigatus*: its role in modulation of conidial morphology and virulence. J Bacteriol 1998; 180: 3031–3038.

170 Ikeda R, Sugita T, Jacobson ES, Shinoda T. Effects of melanin upon susceptibility of *Cryptococcus* to antifungals. Microbiol Immunol 2003; 47: 271–277.

171 Doering TL, Nosanchuk JD, Roberts WK, Casadevall A. Melanin as a potential cryptococcal defence against microbial proteins. Med Mycol 1999; 37: 175–181.

172 Clemens KV, McCusker JH, Davis RW, Stevens DA. Comparative pathogenesis of clinical and nonclinical isolates of *Saccharomyces cerevisiae*. J Infect Dis 1994; 169: 859–867.

173 Byron JK, Clemens KV, McCusker JH, Davis RW, Stevens DA. Pathogenicity of *Saccharomyces cerevisiae* in complement factor five-deficient mice. Infect Immun 1998; 63: 478–485.

174 Kwon-Chung K. Gene disruption to evaluate the role of fungal candidate virulence genes. Curr Opin Microbiol 1998; 1: 381–389.

175 Calderon RA, Fonzi WA. Virulence factors of *Candida albicans*. Trends Microbiol 2001; 9: 327–335.

176 Casadevall A. Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection? Fungal Genet Biol 2005; 42: 98–106.

177 Deepo GS, Jr., Romani L, Calich VL, et al. Knockout mice as experimental models of virulence. Med Mycol 2000; 38(Suppl. 1): S87–S98.

178 Hamilton AJ, Holdom MD. Antioxidant systems in the pathogenic fungi of man and their role in virulence. Med Mycol 1999; 37: 375–389.

179 Haynes K. Virulence in *Candida* species. Trends Microbiol 2001; 9: 591–596.

180 Hogan LH, Klein BS, Levitz SM. Virulence factors of medically important fungi. Clin Microbiol Rev 1996; 9: 469–488.

181 Mitchell AP. Dimorphism and virulence in *Candida albicans*. Genes Genetics of man and their role in virulence. Infect Immun 1998; 66: 375.

182 Navarro-Garcia F, Sanchez M, Nombela C, Pla J. Virulence genes in the pathogenic yeast *Candida albicans*. FEBS Microbiol Rev 2001; 25: 245–268.

183 Odds FC, Gow NA, Brown AJ. Fungal virulence studies come of age. Genome Biol [serial on the internet]. March 2001, 2: REVIEWS 1009.1–1009.4 Available from: http://genomebiology.com/2001/2/3/reviews/1009.1

184 San-Blas G, Travassos LR, Fries BC, et al. Fungal morphogenesis and virulence. Med Mycol 2000; 38(Suppl. 1): S79–S86.

185 Tomee JF, Kauffman HF. Putative virulence factors of *Aspergillus fumigatus*. Clin Exp Allergy 2000; 30: 476–484.

186 Yang YL. Virulence factors of *Candida* species. J Med Microbiol Immunol Infect 2003; 36: 223–228.

187 Laprade L, Boyartchuk VL, Dietrich WF, Winston F. *S. platensis* plays opposite roles in filamentous growth in *Saccharomyces cerevisiae* and *Candida albicans* and is required for *C. albicans* virulence. Genetics 2002; 161: 509–519.

188 Gale CA, Bendel CM, McClellan M, et al. Linkage of adhesion, filamentous growth, and virulence in *Candida albicans* to a single gene, INT1. Science 1998; 279: 1355–1358.

189 Bendel CM, Kinneberg KM, Jechorek RP, et al. Systemic infection following intravenous inoculation of mice with *Candida*
Saccharomyces

190 Braun BR, Johnson AD. TUP1, CPH1 and EFG1 make independent contributions to filamentation in Candida albicans. Genetics 2000; 155: 57–67.

191 Lo HJ, Kohler JR, DiDomenico B, et al. Nonfilamentous C. albicans mutants are avirulent. Cell 1997; 90: 939–949.

192 Rementeria A, Lopez–Molina N, Ludwig A, et al. Genes and molecules involved in Aspergillus fumigatus virulence. Rev Iberoam Micol 2005; 22: 1–23.

193 Mellado E, Auffauvre–Brown A, Gow NA, Holden DW. The Aspergillus fumigatus chsC and chsG genes encode class III chitin synthases with different functions. Mol Microbiol 1996; 20: 667–679.

194 Auffauvre–Brown A, Mellado E, Gow NA, Holden DW. Aspergillus fumigatus chsE: a gene related to CHS3 of Saccharomyces cerevisiae and important for hyphal growth and conidiophore development but not pathogenicity. Fungal Genet Biol 1996; 21: 141–152.

195 Panepinto JC, Oliver BG, Fortwendel JR, et al. Deletion of the Aspergillus fumigatus gene encoding the Ras-related protein RhbA reduces virulence in a model of invasive pulmonary aspergillosis. Infect Immun 2003; 71: 2819–2826.

196 Clemens KV, Miller TK, Selitrennikoff CP, Stevens DA. Fos-1, a putative histidine kinase as a virulence factor for systemic aspergillosis. Med Mycol 2002; 40: 259–262.

197 Langfelder K, Jahn B, Gehringer H, et al. Identification of a polykette synthase gene (pksP) of Aspergillus fumigatus involved in conidial pigment biosynthesis and virulence. Med Microbiol Immunol (Berl) 1998; 187: 79–89.

198 Selitrennikoff CP, Alex L, Miller TK, et al. COS-1, a putative two-component histidine kinase of Candida albicans, is an in vivo virulence factor. Med Mycol 2001; 39: 69–74.

199 Calera JA, Calderone R. Histidine kinase, two-component signal transduction proteins of Candida albicans and the pathogenesis of candidosis. Mycoses 1999; 42(Suppl. 2): S49–S53.

200 Bader T, Bodendorfer B, Schroppel K, Morschehauser J. Calcinurin is essential for virulence in Candida albicans. Infect Immun 2003; 71: 5344–5354.

201 Alsopbaugh JA, Perfect JR, Heitman J. Signal transduction pathways regulating differentiation and pathogenicity of Cryptococcus neoformans. Fungal Genet Biol 1998; 25: 1–14.

202 Steinbach WJ, Cramer RA, Jr., Perfect BZ, et al. Calcinurin controls growth, morphology, and pathogenicity in Aspergillus fumigatus. Eukary Cell 2006; 5: 1091–1103.

203 Cummings CA, Relman DA. Using DNA microarrays to study host–microbe interactions. Emerg Infect Dis 2000; 6: 513–525.

204 Cummings CA, Relman DA. Genomics and microbiology. Microbial forensics – ‘cross-examining pathogens’. Science 2002; 296: 1976–1979.

205 Staub P, Kretschmar M, Nichtein T, et al. Host-induced, stage-specific virulence gene activation in Candida albicans during infection. Mol Microbiol 1999; 32: 533–546.

206 Retallick DM, Depe G, Jr., Woods JP. Applying in vivo expression technology (IVET) to the fungal pathogen Histoplasma capsulatum. Microb Pathog 2000; 28: 169–182.

207 Langfelder K, Philippe B, Jahn B, Latge JP, Brakhage AA. Differential expression of the Aspergillus fumigatus pksP gene detected in vitro and in vivo with green fluorescent protein. Infect Immun 2001; 69: 6411–6418.

208 Goldstein AL, McCusker JH. Development of Saccharomyces cerevisiae as a model pathogen. A system for the genetic identification of gene products required for survival in the mammalian host environment. Genetics 2001; 159: 499–513.

209 Nelson RT, Hua J, Pryor B, Lodge JK. Identification of virulence mutants of the fungal pathogen Cryptococcus neoformans using signature-tagged mutagenesis. Genetics 2001; 157: 935–947.

210 Brown JS, Auffauvre–Brown A, Brown J, et al. Signature-tagged and directed mutagenesis identify PABA synthetase as essential for Aspergillus fumigatus pathogenicity. Mol Microbiol 2000; 36: 1371–1380.

211 Zhang L, Wang M, Li R, Calderone R. Expression of Aspergillus fumigatus virulence-related genes detected in vitro and in vivo with competitive RT–PCR. Mycopathologia 2005; 160: 201–206.

212 Steen BR, Zuyderduyn S, Toffaletti DL, et al. Cryptococcus neoformans gene expression during experimental cryptococcal meningitis. Eukary Cell 2003; 2: 1336–1349.

213 Clemens KV, Stevens DA. Overview of host defense mechanisms in systemic mycoses and the basis for immunotherapy. Semin Respir Infect 2001; 16: 60–66.

214 Romani L. Immunity to fungal infections. Nat Rev Immunol 2004; 4: 1–23.

215 Chang YC, Segal BH, Holland SM, Miller GF, Kwon-Chung KJ. Virulence of catalase-deficient Aspergillus nidulans in p47(phox)–/– mice. Implications for fungal pathogenicity and host defense in chronic granulomatous disease. J Clin Invest 1998; 101: 1843–1850.

216 Perfect JR, Wong B, Chang YC, Kwon-Chung KJ, Williamson PR. Cryptococcus neoformans: virulence and host defences. Med Mycol 1998; 36(Suppl. 1): S79–S86.

217 Cheers C, Waller R. Activated macrophages in congenitally athymic ‘nude mice’ and in lethally irradiate mice. J Immunol 1975; 115: 844–847.

218 Cutler JE. Acute systemic candidiasis in normal and congenitally thymic-deficient (nude) mice. J Reticuloendothel Soc 1976; 19: 121–124.

219 Huffnagle GB, Depe G. Innate and adaptive determinants of host susceptibility to medically important fungi. Curr Opin Microbiol 2003; 6: 344–350.

220 Zaas AK. Host genetics affect susceptibility to invasive aspergillosis. Med Mycol 2006; 44(Suppl. 1): S55–S60.

221 Hector RF, Domer JE, Carrow EW. Immune responses to Candida albicans in genetically distinct mice. Infect Immun 1982; 38: 1020–1028.

222 Hector RF, Yee E, Collins MS. Use of DBA/2N mice in models of systemic candidiasis and pulmonary and systemic aspergillosis. Infect Immun 1990; 58: 1476–1478.

223 Kirkland TN, Fierer J. Inbred mouse strains differ in resistance to lethal Coccidioides immitis infection. Infect Immun 1983; 40: 912–916.

224 Madan T, Reid KB, Singh M, Sarma PU, Kishore U. Susceptibility of mice genetically deficient in the surfactant protein (SP-)A or SP-D gene to pulmonary hypersensitivity induced by antigens and allergens of Aspergillus fumigatus. J Immunol 2005; 174: 6943–6954.

225 Lassag C, Kolb A, Strobl B, Enjuanes L, Muller M. Studying human pathogens in animal models: fine tuning the humanized mouse. Transgenic Res 2005; 14: 803–806.

226 Hohl TM, Rivera A, Pamer EG. Immunity to fungi. Curr Opin Immunol 2006; 18: 465–472.

227 Kickberg BJ. Trends in immunotherapy of fungal infections. Eur J Clin Microbiol Infect Dis 1997; 16: 51–55.

228 Stevens DA, Walsh TJ, Bistoni F, et al. Cytokines and mycoses. Med Mycol 1998; 36(Suppl. 1): S174–S182.
et al

284 Murphy JW, Hidore MR, Nabavi N. Binding interactions of Marconi P, Scaringi L, Tissi L, Tapping RI, Akashi S, Miyake K, Godowski PJ, Tobias PS. Toll-like receptor-4, but not toll-like receptor 2, is a signaling receptor for fungal asthma in a murine model; putative involvement of Toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis. J Infect Dis 1996; 165: 3613.

285 Arruda C, Franco MF, Kashino SS, et al. Interleukin-12 protects mice against disseminated infection caused by Paracoccidioides brasiliensis but enhances pulmonary inflammation. Clin Immunol 2002; 103: 185–195.

286 Kawakami K, Shibuya K, Qureshi MH, et al. Chemokine responses and accumulation of inflammatory cells in the lungs of mice infected with highly virulent Cryptococcus neoformans: effects of interleukin-12. FEMS Immunol Med Microbiol 1999; 25: 391–402.

287 Magee DM, Cox RA. Interleukin-12 regulation of host defenses against Coccidioides immitis. Infect Immun 1996; 64: 3609–3613.

288 Stevens DA. Th1/Th2 in aspergillosis. Med Mycol 2000; 44(Suppl. 1): S229–S235.

289 Romani L, Bistoni F, Puccetti P. Initiation of T-helper cell immunity to Candida albicans by IL-12: the role of neutrophils. Chem Immunol 1997; 68: 110–135.

290 Cain JA, Deepe GS, Jr. Interleukin-12 neutralization alters lung inflammation and leukocyte expression of CD80, CD86, and major histocompatibility complex class II in mice infected with Histoplasma capsulatum. Infect Immun 2000; 68: 2069–2076.

291 Rayhan E, Lortholary O, Fitting C, et al. Enhanced sensitivity of tumor necrosis factor/lymphotxin-z-deficient mice to Cryptococcus neoformans infection despite increased levels of nitrite/nitrate, interferon-γ, and interleukin-12. J Infect Dis 1999; 180: 1637–1647.

292 Kawakami K, Shibuya K, Qureshi MH, et al. Chemokine responses and accumulation of inflammatory cells in the lungs of mice infected with highly virulent Cryptococcus neoformans: effects of interleukin-12. FEMS Immunol Med Microbiol 1999; 25: 391–402.

293 Magee DM, Cox RA. Interleukin-12 regulation of host defenses against Coccidioides immitis. Infect Immun 1996; 64: 3609–3613.

294 Arruda C, Franco MF, Kashino SS, et al. Interleukin-12 protects mice against disseminated infection caused by Paracoccidioides brasiliensis but enhances pulmonary inflammation. Clin Immunol 2002; 103: 185–195.

295 Moore KW, O’Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol 1993; 11: 165–190.

296 Rennick DM, Fort MM, Davidson NJ. Studies with IL-10-/- mice: an overview. J Leukoc Biol 1997; 61: 389–396.

297 Clemons KV, Grunig G, Sobel RA, et al. Studies with IL-10-/- mice: an overview. J Leukoc Biol 1997; 61: 389–396.

298 Netea MG, Van Der Graaf CA, Vonk AG, et al. Role of Toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis. J Infect Dis 2002; 185: 1483–1489.

299 Villamon E, Gozalbo D, Roig P, et al. Toll-like receptor-2 is essential in murine defenses against Candida albicans infections. Microbes Infect 2004; 6: 1–7.

300 Lekkala M, LeVine AM, Linke MJ, et al. Surfactant protein-D and pulmonary host defense. Crouch EC. Surfactant protein-D and pulmonary host defense. Respir Res 2000; 1: 93–108.

301 Mencacci A, Cenci E, Del Sero G, et al. Induction of natural killer cell activity by inactivated Candida albicans in mice. Infect Immun 1985; 50: 297–303.

302 Rennick DM, Fort MM, Davidson NJ. Studies with IL-10-/- mice: an overview. J Leukoc Biol 1997; 61: 389–396.

303 Rennick DM, Fort MM, Davidson NJ. Studies with IL-10-/- mice: an overview. J Leukoc Biol 1997; 61: 389–396.

304 Murphy JW, Hidore MR, Nabavi N. Binding interactions of murine natural killer cells with the fungal target Cryptococcus neoformans. Infect Immun 1991; 59: 1476–1488.

305 Lipscombok MF, Alvarellos T, Toews GB, et al. Role of natural killer cells in resistance to Cryptococcus neoformans infections in mice. Am J Pathol 1987; 128: 354–361.

306 Kawakami K, Koguchi Y, Qureshi MH, et al. NK cells eliminate Cryptococcus neoformans by potentiating the fungidal activity of macrophages rather than by directly killing them upon stimulation with IL-12 and IL-18. Microbiol Immunol 2000; 44: 1043–1050.

307 Kawakami K, Koguchi Y, Qureshi MH, et al. IL-18 contributes to host resistance against infection with Cryptococcus neoformans in mice with defective IL-12 synthesis through induction of IFN-γ production by NK cells. J Immunol 2000; 165: 941–947.

308 Stevens DA. Th1/Th2 in aspergillosis. Med Mycol 2006; 44(Suppl. 1): S229–S235.

309 Romani L, Bistoni F, Puccetti P. Initiation of T-helper cell immunity to Candida albicans by IL-12: the role of neutrophils. Chem Immunol 1997; 68: 110–135.

310 Cain JA, Deepe GS, Jr. Interleukin-12 neutralization alters lung inflammation and leukocyte expression of CD80, CD86, and major histocompatibility complex class II in mice infected with Histoplasma capsulatum. Infect Immun 2000; 68: 2069–2076.

311 Rayhan E, Lortholary O, Fitting C, et al. Enhanced sensitivity of tumor necrosis factor/lymphotxin-z-deficient mice to Cryptococcus neoformans infection despite increased levels of nitrite/nitrate, interferon-γ, and interleukin-12. J Infect Dis 1999; 180: 1637–1647.

312 Kawakami K, Shibuya K, Qureshi MH, et al. Chemokine responses and accumulation of inflammatory cells in the lungs of mice infected with highly virulent Cryptococcus neoformans: effects of interleukin-12. FEMS Immunol Med Microbiol 1999; 25: 391–402.

313 Magee DM, Cox RA. Interleukin-12 regulation of host defenses against Coccidioides immitis. Infect Immun 1996; 64: 3609–3613.

314 Arruda C, Franco MF, Kashino SS, et al. Interleukin-12 protects mice against disseminated infection caused by Paracoccidioides brasiliensis but enhances pulmonary inflammation. Clin Immunol 2002; 103: 185–195.

315 Moore KW, O’Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol 1993; 11: 165–190.

316 Rennick DM, Fort MM, Davidson NJ. Studies with IL-10-/- mice: an overview. J Leukoc Biol 1997; 61: 389–396.

317 Clemons KV, Grunig G, Sobel RA, et al. Studies with IL-10-/- mice: an overview. J Leukoc Biol 1997; 61: 389–396.
304 Hurst SD, Muchamuel T, Gorman DM, et al. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002; **169**: 443–453.

305 Rivera J, Zaragoza O, Casadevall A. Antibody-mediated protection against *Cryptococcus neoformans* pulmonary infection is dependent on B cells. *Infect Immun* 2005; **73**: 1141–1150.

306 Casadevall A, Pirofski L. Insights into mechanisms of antibody-mediated immunity from studies with *Cryptococcus neoformans*. *Curr Mol Med* 2005; **5**: 421–433.

307 Magee DM, Friedberg RL, Woitaske MD, Johnston SA, Cox RA. Role of B cells in vaccine-induced immunity against coccidiodiomycosis. *Infect Immun* 2005; **73**: 7011–7013.

308 Mandi Y, Forkas G, Osoczyvski I. Effects of pentoxifyllin and PentaglobinO on TNF and IL-6 production in septic patients. *Acta Microbiol Immunol Hung* 1995; **42**: 301–308.

309 Hunter RL. Overview of vaccine adjuvants: present and future. *Vaccine* 2002; **20**(Suppl. 3): S7–S12.

310 Kullberg BJ, Netea MG, Curfs JH, et al. Combined murine granulocyte colony-stimulating factor protects against acute disseminated *Candida albicans* infection in nonneutropenic mice. *J Infect Dis* 1998; **177**: 175–181.

311 Stevens DA, Domer JE, Ashman RB, Blackstock R, Brummer E. Immunomodulation in mycoses. *J Med Vet Mycol* 1994; **32**(Suppl. 1): S253–S265.

312 Stevens DA, Kullberg BJ, Brummer E, et al. Combined treatment: antifungal drugs with antibodies, cytokines or drugs. *Med Mycol* 2000; **38**(Suppl. 1): S305–S315.

313 Mencacci A, Cenci E, Bacci A, Bistoni F, Romani L. Host immune reactivity determines the efficacy of combination immunotherapy and antifungal chemotherapy in candidiasis. *J Infect Dis* 2000; **181**: 686–694.

314 Van’t Wout JW, Van der Meer JW, Barza M, Dinarello CA. Protection of neutrophic mice from lethal candida albicans infection by recombinant interleukin 1. *Eur J Immunol* 1988; **18**: 1143–1146.

315 Cano LE, Kashino SS, Arruda C, et al. Protective role of gamma interferon in experimental pulmonary paracoccidioidomycosis. *Infect Immun* 1998; **66**: 800–806.

316 Clemons KV, Lutz JE, Stevens DA. Efficacy of recombinant gamma interferon for treatment of systemic cryptococcosis in SCID mice. *Antimicrob Agents Chemother* 2001; **45**: 686–689.

317 Clemons KV, Lutz JE, Stevens DA. Efficacy of interferon-γ and amphotericin B for the treatment of systemic murine histoplasmosis. *Microbes Infect* 2001; **3**: 3–10.

318 Hostetter JS, Brummer E, Cofman RL, Stevens DA. Effect of antifungal triazole and antifungal triazole (SCH 42427) in paracoccidioidomycosis: correlation of IgE levels with outcome. *Clin Exp Immunol* 1993; **94**: 11–16.

319 Joly V, Saint-Julien L, Carbon C, Yeni P. In vivo activity of interferon-γ in combination with amphotericin B in the treatment of experimental cryptococcosis. *J Infect Dis* 1994; **170**: 1331–1334.

320 Kawakami K, Kohno S, Kadota J, et al. T cell-dependent activation of macrophages and enhancement of their phagocytic activity in the lungs of mice inoculated with heat-killed *Cryptococcus neoformans*: involvement of IFN-γ and its protective effect against cryptococcal infection. *Microbiol Immunol* 1995; **39**: 135–143.

321 Kawakami K, Tohyama M, Teruya K, et al. Contribution of interferon-γ in protecting mice during pulmonary and disseminated infection with *Cryptococcus neoformans*. *FEMS Immunol Med Microbiol* 1996; **13**: 123–130.

322 Kullberg BJ, van’t Wout JW, Hoogstraten C, van Furth R. Recombinant interferon-γ enhances resistance to acute disseminated *Candida albicans* infection in mice. *J Infect Dis* 1993; **168**: 436–443.

323 Rex HJ, Bennett JE, Gallin JI, et al. In vivo interferon-γ therapy augments the in vitro ability of chronic granulomatous disease neutrophils to damage Aspergillus hyphae. *J Infect Dis* 1991; **163**: 849–852.

324 Stevens DA, Brummer E, Clemons KV. Interferon-γ as an antifungal. *J Infect Dis* 2006; **194**(Suppl. 1): S33–S37.

325 Clemons KV, Brummer E, Stevens DA. Cytokine treatment of central nervous system infection: efficacy of interleukin-12 alone and synergy with conventional antifungal therapy in experimental cryptococcosis. *Antimicrob Agents Chemother* 1994; **38**: 460–464.

326 Kawakami K, Qureshi MH, Koguchi Y, Nakajima K, Saito A. Differential effect of *Cryptococcus neoformans* on the production of IL-12p40 and IL-10 by murine macrophages stimulated with lipopolysaccharide and gamma interferon. *FEBS Microbiol Lett* 1999; **175**: 87–94.

327 Kawakami K, Qureshi MH, Zhang T, et al. IL-18 protects mice against pulmonary and disseminated infection with *Cryptococcus neoformans* by inducing IFN-γ production. *J Immunol* 1997; **159**: 5528–5534.

328 Kawakami K, Tohyama M, Xie Q, Saito A. IL-12 protects mice against pulmonary and disseminated infection caused by *Cryptococcus neoformans*. *Clin Exp Immunol* 1996; **104**: 208–214.

329 Kobayashi M, Takahashi H, Herndon DN, Pollard RB, Suzuki F. Effect of a combination therapy between IL-12 and soluble IL-4 receptor (sIL-4R) on *Candida albicans* and herpes simplex virus type I infections in thermally injured mice. *Can J Microbiol* 2002; **48**: 886–894.

330 Lavigne LM, Schopf LR, Chung CL, Maylor R, Sypek JP. The role of recombinant murine IL-12 and IFN-γ in the pathogenesis of a murine systemic *Candida albicans* infection. *J Immunol* 1998; **160**: 284–292.

331 Zhou P, Sieve MC, Bennett J, et al. IL-12 prevents mortality in mice infected with *Histoplasma capsulatum* through induction of IFN-γ. *J Immunol* 1995; **155**: 785–795.

332 Zhou P, Sieve MC, Tewari RP, Seder RA. Interleukin-12 modulates the protective immune response in SCID mice infected with *Histoplasma capsulatum*. *Infect Immun* 1997; **65**: 936–942.

333 Brummer E, Hanson LH, Stevens DA. IL-4, IgE, and interferon-γ production in pulmonary blastomycosis: comparison in mice untreated, immunized, or treated with an antifungal (SCH 39304). *Cell Immunol* 1993; **149**: 258–267.

334 Ortoneda M, Capilla J, Pujol I, et al. Liposomal amphotericin B and granulocyte colony-stimulating factor therapy in a murine model of invasive infection by *Scedosporium prolificans*. *J Antimicrob Chemother* 2002; **49**: 525–529.

335 Vonk AG, Netea MG, van Krieken JH, et al. Treatment of intra-abdominal abscesses caused by *Candida albicans* with antifungal agents and recombinant murine granulocyte colony-stimulating factor. *Antimicrob Agents Chemother* 2003; **47**: 3688–3693.

336 Graybill JR, Bocanegra R, Lambros C, Luther MF. Granulocyte colony stimulating factor therapy of experimental cryptococcal meningitis. *J Med Vet Mycol* 1997; **35**: 243–247.

337 Yamamoto Y, Uchida K, Klein TW, Friedman H, Yamaguchi H. Immunomodulators and fungal infections: use of antifungal drugs in combination with G-CSF. *Adv Exp Med Biol* 1992; **319**: 231–241.

© 2007 ISHAM, Medical Mycology, **45**, 657–684.
338 Vitt CR, Fidler JM, Ando D, Zimmerman RJ, Au kernan SL. Antifungal activity of recombinant human macrophage colony-stimulating factor in models of acute and chronic candidiasis in the rat. *J Infect Dis* 1994; 169: 369–374.

339 Deepe GS, Jr., Gibbons R. Recombinant murine granulocyte-macrophage colony-stimulating factor modulates the course of pulmonary histoplasmosis in immunocompetent and immunodeficient mice. *Antimicrob Agents Chemother* 2000; 44: 3328–3336.

340 Sionov E, Mendlovic S, Segal E. Experimental systemic murine aspergillosis: treatment with polyclone and caspofungin combination and G-CSF. *J Antimicrob Chemother* 2005; 56: 594–597.

341 Polak-Wyss A. Protective effect of human granulocyte colony-stimulating factor (hG-CSF) on *Cryptococcus* and *Aspergillus* infections in normal and immunosuppressed mice. *Mycoses* 1991; 34: 205–215.

342 Casadevall A, Pirofsk LA. Feasibility and prospects for a vaccine to prevent cryptococcosis. *Med Mycol* 2005; 43: 667–680.

343 Dan JM, Levit SM. Prospects for development of vaccines against fungal diseases. *Drug Resist Updat* 2006; 9: 105–110.

344 Datta K, Pirofski LA. Towards a vaccine for *Cryptococcus neoformans*: principles and caveats. *FEBS Yeast Res* 2006; 6: 525–536.

345 Deepe GS, Jr., Wuthrich M, Klein BS. Progress in vaccination for histoplasmosis and blastomycosis: coping with cellular immunity. *Med Mycol* 2005; 43: 381–389.

346 Dixon DM, Casadevall A, Klein B, et al. Development of vaccines and their use in the prevention of fungal infections. *Med Mycol* 1998; 36(Suppl 1): S57–S67.

347 Feldmesser M. Prospects of vaccines for invasive aspergillosis. *Med Mycol* 2005; 43: 571–587.

348 Lehmann PF, White LO. Acquired immunity to *Aspergillus fumigatus*. *Infect Immun* 1976; 13: 1296–1298.

349 Fromtling RA, Blackstock R, Hall NK, Bulmer GS. Immunization of mice to *Aspergillus* like mutants of *Cryptococcus neoformans*. *Mycopathologia* 1979; 68: 179–181.

350 Fromtling RA, Kaplan AM, Shadomy HJ. Immunization of mice with stable, acapsular, yeast–like mutants of *Cryptococcus neoformans*. *Sabouraudia* 1983; 21: 113–119.

351 Levine HB, Cobb JM, Smith CE. Immunogenicity of spherule-endospore vaccines of *Coccidioides immitis* for mice. *J Immunol* 1961; 87: 218–227.

352 Levine HB, Kong YC, Smith C. Immunization of mice to *Coccidioides immitis* dose, regimen and spherulation stage of killed spherule vaccines. *J Immunol* 1965; 94: 132–142.

353 Levine HB, Kong YC. Immunologic impairment in mice treated intravenously with killed *Coccidioides immitis* spherules: suppressed response to intramuscular doses. *J Immunol* 1966; 97: 297–305.

354 Pappagianis D. Evaluation of the protective efficacy of the killed *Coccidioides immitis* spherule vaccine in humans. The Valley Fever Vaccine Study Group. *Am Rev Respir Dis* 1993; 148: 656–660.

355 Cole GT, Xue JM, Okeke CN, et al. A vaccine against coccidioidomycosis is justified and attainable. *Med Mycol* 2004; 42: 189–216.

356 Cox RA, Magee DM. Coccidioidomycosis: host response and vaccine development. *Clin Microbiol Rev* 2004; 17: 804–839.

357 Delgado N, Xue J, Yu JJ, Hung CY, Cole GT. A recombinant β-1,3-glucanotransferase homolog of *Coccidioides posadasi* protects mice against coccidioidomycosis. *Infect Immun* 2003; 71: 3010–3019.

358 Xue J, Hung CY, Yu JJ, Cole GT. Immune response of vaccinated and non-vaccinated mice to *Coccidioides posadasi* infection. *Vaccine* 2005; 23: 3535–3544.

359 Awasthi S, Awasthi V, Magee DM, Coalson JJ. Efficacy of antigen 2/proline-rich antigen cDNA-transfected dendritic cells in immunization of mice against *Coccidioides posadasi*. *J Immunol* 2005; 175: 3900–3906.

360 Peng T, Shubitz L, Simons J, et al. Localization within a proline-rich antigen (Ag2/PRA) of protective antigenity against infection with *Coccidioides immitis* in mice. *Infect Immun* 2002; 70: 3330–3335.

361 Bozza S, Gaziano R, Lipford GB, et al. Vaccination of mice against invasive aspergillosis with recombinant *Aspergillus* proteins and CpG oligodeoxynucleotides as adjuvants. *Microbes Infect* 2002; 4: 1281–1290.

362 Kirkland TN, Finley F, Orsborn KJ, Galgiani JN. Evaluation of the proline-rich antigen of *Coccidioides immitis* as a vaccine candidate in mice. *Infect Immun* 1998; 66: 3519–3522.

363 Ito S, Pedras-Vasconcelos J, Klinman DM. CpG oligodeoxynucleotides increases the susceptibility of normal mice to infection by *Candida albicans*. *Infect Immun* 2005; 73: 6154–6156.

364 Polonelli L, Casadevall A, Han Y, et al. The efficacy of acquired humoral and cellular immunity in the prevention and therapy of experimental fungal infections. *Med Mycol* 2000; 38(Suppl 1): S281–S292.

365 Chan CM, Woo PC, Leung AS, et al. Detection of antibodies specific to an antigenic cell wall galactomannoprotein for serodiagnosis of *Aspergillus fumigatus* aspergillosis. *J Clin Microbiol* 2002; 40: 2041–2045.

366 Centeno-Lima S, de Lacerda JM, do Carmo JA, et al. Follow-up of anti-*Aspergillus* IgG and IgA antibodies in bone marrow transplanted patients with invasive aspergillosis. *Clin Lab Anal* 2002; 16: 156–162.

367 Chow LP, Liu SL, Yu CJ, et al. Identification and expression of an allergen Asp f 13 from *Aspergillus fumigatus* fumigatus and epitope mapping using human IgE antibodies and rabbit polyclonal antibodies. *Biochem J* 2000; 346(Pt 2): 423–431.

368 Rydjord B, Hetland G, Wiker HG. Immunoglobulin G antibodies against environmental moulds in a Norwegian healthy population shows a bimodal distribution for *Aspergillus versicolor*. *Scand J Immunol* 2005; 62: 281–288.

369 Han Y, Cutler JE. Antibody response that protects against disseminated candidiasis. *Infect Immun* 1995; 63: 2714–2719.

370 Mukherjee J, Feldmesser M, Scharff MD, Casadevall A. Monoclonal antibodies to *Cryptococcus neoformans* glucuronoxylomannan enhance fluconazole efficacy. *Antimicrob Agents Chemother* 1995; 39: 1398–1405.

371 Devi SJ. Preclinical efficacy of a glucuronoxylomannan-tetanus toxoid conjugate vaccine of *Cryptococcus neoformans* in a murine model. *Vaccine* 1996; 14: 841–844.

372 Torosantucci A, Bromuro C, Chiania P, et al. A novel glycol-conjugate vaccine against fungal pathogens. *J Exp Med* 2005; 202: 597–606.

373 Stevens DA. Vaccinate against aspergillosis! A call to arms of the immune system. *Clin Infect Dis* 2004; 38: 1131–1136.

374 Ito JI, Lyons JM. Vaccination of corticosteroid immunosuppressed mice against invasive pulmonary aspergillosis. *J Infect Dis* 2002; 186: 869–871.

375 Wuthrich M, Filutowicz HI, Warner T, Deepe GS, Jr., Klein BS. Vaccine immunity to pathogenic fungi overcomes the requirement for CD4 help in exogenous antigen presentation to CD8+ T cells: implications for vaccine development in immune-deficient hosts. *J Exp Med* 2003; 197: 1405–1416.
376 Fierer J, Waters C, Walls L. Both CD4+ and CD8+ T cells can mediate vaccine-induced protection against Coccidioides immitis infection in mice. J Infect Dis 2006; 193: 1323–1331.

377 Walsh TJ, Groll A, Hiemenz J, et al. Infections due to emerging and uncommon medically important fungal pathogens. Clin Microbiol Infect 2004; 10(Suppl 1): S48–S66.

378 Walsh TJ, Groll AH. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. Transpl Infect Dis 1999; 1: 247–261.

379 Steinbach WJ, Stevens DA, Denning DW. Combination and sequential antifungal therapy for invasive aspergillosis: review of published in vitro and in vivo interactions and 6281 clinical cases from 1966 to 2001. Clin Infect Dis 2003; 37(Suppl 3): S188–S224.

380 Walsh TJ, Viviani MA, Arathoon E, et al. New targets and delivery systems for antifungal therapy. Med Mycol 2000; 38(Suppl 1): S335–S347.

381 Boucher HW, Groll AH, Chiou CC, Walsh TJ. Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. Drugs 2004; 64: 1997–2020.

382 Wingard JR, Leather H. A new era of antifungal therapy. Biol Blood Marrow Transplant 2004; 10: 73–90.

383 Fuhr U. Drug interactions with grapefruit juice. Extent, probable mechanism and clinical relevance. Drug Saf 1998; 18: 251–272.

384 Sugar AM, Liu XP. Effect of grapefruit juice on serum voriconazole concentrations in the mouse. Med Mycol 2000; 38: 209–212.

385 Clemons KV, Espiritu M, Parmar R, Stevens DA. Comparative efficacies of conventional amphotericin B, liposomal amphotericin B (AmBisome), caspofungin, micafungin, and voriconazole alone or in combination against systemic murine central nervous system aspergillosis. Antimicrob Agents Chemother 2005; 49: 4867–4875.

386 Luque JC, Clemons KV, Stevens DA. Efficacy of micafungin alone or in combination against systemic murine aspergillosis. Antimicrob Agents Chemother 2003; 47: 1452–1455.

387 Clemons KV, Stevens DA. Efficacy of micafungin alone or in combination against experimental pulmonary aspergillosis. Med Mycol 2006; 44: 69–73.

388 Wiederhold NP, Tam VH, Chi J, et al. Pharmacodynamic activity of amphotericin B deoxycholate is associated with peak plasma concentrations in a neutropenic murine model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 2006; 50: 469–473.

389 Clemons KV, Parmar R, Martinez M, Stevens DA. Efficacy of Abelcet alone, or in combination therapy, against experimental central nervous system aspergillosis. J Antimicrob Chemother 2006; 58: 466–469.

390 Schinabeck MK, Long LA, Hossain MA, et al. Rabbit model of Candida albicans biofilm infection: liposomal amphotericin B antifungal lock therapy. Antimicrob Agents Chemother 2004; 48: 1727–1732.

391 Mukherjee PK, Mohamed S, Chandra J, et al. Alcohol dehydrogenase restricts the ability of the pathogen Candida albicans to form a biofilm on catheter surfaces through an ethanol-based mechanism. Infect Immun 2006; 74: 3804–3816.

392 Stevens DA. Combination immunotherapy and antifungal chemotherapy. Clin Infect Dis 1998; 26: 1266–1269.

393 Dodds ES, Drew RH, Perfect JR. Antifungal pharmacodynamics: review of the literature and clinical applications. Pharmacother 2000; 20: 1335–1355.

394 Andes D. Clinical pharmacodynamics of antifungals. Infect Dis Clin North Am 2003; 17: 635–649.

395 Andes D. Clinical utility of antifungal pharmacokinetics and pharmacodynamics. Curr Opin Infect Dis 2004; 17: 533–540.

396 Hosseini-Yeganeh M, McLachlan AJ. Physiologically based pharmacokinetic model for terbinafine in rats and humans. Antimicrob Agents Chemother 2002; 46: 2219–2228.

397 Grosso DS, Boyden TW, Pamenter RW, et al. Ketoconazole inhibition of testicular secretion of testosterone and displacement of steroid hormones from serum transport proteins. Antimicrob Agents Chemother 1983; 23: 207–212.

398 Clemons KV, Sobel RA, Stevens DA. Toxicity of LY303366, an echinocandin antifungal, in mice pretreated with glucocorticoids. Antimicrob Agents Chemother 2000; 44: 378–381.

399 Clemons KV, Sobel RA, Williams PL, Stevens DA. Comparative toxicities and pharmacokinetics of intrathecal lipid (amphotericin B colloidal dispersion) and conventional deoxycholate formulations of amphotericin B in rabbits. Antimicrob Agents Chemother 2001; 45: 612–615.

400 Clemons KV, Ranney DF, Stevens DA. A novel heparin-coated hydrophilic preparation of amphotericin B hydrosomes. Curr Opin Investig Drugs 2001; 2: 480–487.

401 Francis P, Lee JW, Hoffman A, et al. Efficacy of unilamellar liposomal amphotericin B in treatment of pulmonary aspergillosis in persistently granulocytopenic rabbits: the potential role of bronchoalveolar D-mannitol and serum galactomannan as markers of infection. J Infect Dis 1994; 169: 356–368.

402 Dupont B, Huber M, Kim SJ, Bennett JE. Galactomannan antigenemia and antigenuria in aspergillosis: studies in patients and experimentally infected rabbits. J Infect Dis 1987; 155: 1–11.

403 Loeffler J, Kloepfer K, Hebart H, et al. Polymerase chain reaction detection of Aspergillus DNA in experimental models of invasive aspergillosis. J Infect Dis 2002; 185: 1203–1206.

404 Marr KA, Balajee SA, McLaughlin L, et al. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. J Infect Dis 2004; 190: 641–649.

405 Becker MJ, de Marie S, Willemse D, Bakker-Woudenberg IA. Quantitative galactomannan detection is superior to PCR in diagnosing and monitoring invasive pulmonary aspergillosis in an experimental rat model. J Clin Microbiol 2000; 38: 1434–1438.

406 Hurst SF, Reyes GH, McLaughlin DW, Reiss E, Morrison CJ. Comparison of commercial latex agglutination and sandwich enzyme immunoassays with a competitive binding inhibition enzyme immunoassay for detection of antigenemia and antigenuria in a rabbit model of invasive aspergillosis. Clin Diagn Lab Immunol 2000; 7: 477–485.

407 Segal BH, Barnhart LA, Anderson VL, et al. Posaconazole as salvage therapy in patients with chronic granulomatous disease and invasive filamentous fungal infection. Clin Infect Dis 2005; 40: 1684–1688.

408 Verweij PE. Advances in diagnostic testing. Med Mycol 2005; 43(Suppl 1): S121–S124.

409 Goldman D, Lee SC, Casadevall A. Pathogenesis of pulmonary Cryptococcus neoformans infection in the rat. Infect Immun 1994; 62: 4755–4761.

410 Wong B, Perfect JR, Beggs S, Wright KA. Production of the hexitol D-mannitol by Cryptococcus neoformans in vitro and in rabbits with experimental meningitis. Infect Immun 1990; 58: 1664–1670.