Introduction

For many years, medical mycology was considered to be an esoteric discipline whose significance was usually made relevant by clinicians who were accidentally or specifically interested in ‘exotic’ infections amongst their patients. Over the past 10 or 20 years, research on the biology of medically important fungi has increased and, more recently, entered into an era of genomics for at least three reasons, as follows. First, people die of fungal infections in industrialized countries. *Candida* species are, for example, fourth in frequency among all microorganisms isolated from blood samples in US hospitals [1,2]. One in 25 patients who die in European teaching hospitals have invasive aspergillosis [3]. The number of deaths due to fungi largely surpasses that due to parasites in developed countries.

Second, antifungal drugs are profitable for pharmaceutical companies, a clear indication that these antimicrobial agents are needed. Complicating the issue of drug therapy has been the emergence of drug resistance. This has resulted, for example, in the change in the *Candida* species profile among patients with an increase in percentage of non-*C. albicans* species [4]. This change in species profile among patients is primarily associated with their drug resistance.

Third, sequencing of the genomes of most human pathogens (*Candida glabrata, C. albicans, Aspergillus fumigatus* and *Cryptococcus neoformans*) is almost complete, and sequencing of other genomes is underway or projected (*Coccidioides, Pneumocystis, Histoplasma, Paracoccidioides* and *Trichophyton*), exceeding in number those of phytopathogenic or ‘model’ fungi, such as *Saccharomyces cerevisiae*.

Most recent research efforts, highlighted by the reviews in this Host–microbe interactions: fungi section, are focused on the analysis of the physiopathology of invasive fungal infections (disseminated infections are the critical ones, as they kill patients), the understanding of fungal virulence factors as well as the problems associated with susceptibility to infection as a result of abrogated host immunity or resistance to antifungal drugs. It is especially important for readers of this section to remember two points. First, opportunistic infections caused by commensal (*Candida*) or fully saprophytic (*Aspergillus*) species almost exclusively invade the immunocompromised hosts. The opportunistic pathogens are normally eradicated by the innate immunity of the immunocompetent host. Immunosuppression is indeed the key factor that triggers the establishment of disseminated fungal infections. For example, a 20 g immunocompetent mouse can survive inhalation of $10^6$ conidia of *A. fumigatus*, whereas a 20 kg human-bone-marrow-transplant patient who inhales 100 conidia per day will be at risk of invasive aspergillosis (IA). Most interestingly, it is the type and severity of the immunosuppressive conditions associated with the biological properties of the fungus that will determine which fungal species will invade the host. For example, chemo- and radiotherapies for leukemia treatment or organ transplantation are associated with *Aspergillus* infections, whereas HIV infection is more often associated with
C. albicans, Pneumocystis carinii or C. neoformans infections and, in fact, the latter group of diseases are referred to as AIDS-defining diseases [5].

Second, it is important to understand that fungi do not cause the same diseases and, like the bacterial pathogens, the pathobiology of each disease varies with the species.

**Pathobiology of invasive mycoses**

The establishment of an infection by a fungal pathogen and its invasion and growth in host tissues requires that the fungus is aggressive at a time when the immune host response is debilitated. This cause/effect relationship of fungus–host interactions is discussed in the reviews by Mansour and Levitz (pp 359–365) and Gow, Brown and Odds (pp 366–371).

In their review, Mansour and Levitz list the mechanisms used by phagocytes from the immunocompetent host to destroy the fungal intruders. It is clear that a coordinated immune response is essential for fungal clearance. In general, the macrophage serves as the central cell of the innate immune system, whereas dendritic cells and neutrophils play roles in immunity either before (dendritic cells) or after (neutrophils) macrophage activity. This latter statement is especially true for inhaled fungal pathogens, but exceptions to the rule exist. For example, neutrophils probably play a primary role in eradicating blood-borne Candida species. Obviously, cytokines and chemokines play an important regulatory role in inhibiting or stimulating the phagocytic response. A knowledge of the phagocytic response of the immunocompetent host is a prerequisite to the identification of the key host factors that are reduced by immunosuppression. These factors, in turn, lead to fungal development and invasion. This information will explain the specificity of the fungal infections that is associated with the differential reactivities of the immune system towards each fungal species. However, there are currently little data on the human immune response and, importantly but not surprisingly, it is known that mouse and human immune responses towards fungi can be different. Understanding the immune response of the human host will be a real challenge, especially considering that it is ethically unreasonable and difficult to obtain cells from patients at risk for these infections. Another interesting observation is the evidence that almost every human cell in contact with the fungal invaders (dendritic cells, macrophages, neutrophils, epithelial and endothelial cells; see [6] and the review by Mansour and Levitz) is able to engulf yeasts or conidia. Short- or long-term survival and intracellular modification of these phagocytosed forms have not been investigated enough, and the engulfed fungal elements could represent a latent inoculum that can be activated by any immune or therapeutic perturbation. Fungal infections due to the relapse of a dormant inoculum have been suggested in cases of chronic pulmonary histoplasmosis (see, for example, [7]).

Gow, Brown and Odds discuss the paradigm of morphogenesis and fungal virulence, most of which has been drawn from studies on the polymorphism of one of the predominant fungal pathogens, C. albicans. They postulate that conversion of unicellular yeast forms to filamentous growth is essential for virulence of C. albicans. Morphogenesis by itself is under the control of multiple sensing mechanisms and signal transduction pathways. The authors stress, however, that even though filamentous growth forms have advantages over yeast in penetrating a cell or a tissue, a unicellular yeast phase is the preferred tissue phase of dimorphic fungal pathogens such as Histoplasma capsulatum. Morphogenesis is only one phenotype that is controlled positively or negatively by a network of cross-talking signal pathways, of which the Ras-cAMP/MAP kinase pathway is probably the most important [8,9]. Understanding the kinetics of these global regulators during the different phases of infection, aside from focusing on morphogenesis, should reveal new virulence traits of human pathogens.

Correlating morphogenesis or any biological property with virulence remains largely based on animal studies. Analysis of fungal virulence is hampered by the inadequacy of most animal models compared to human disease, as stated above. Furthermore, an equilibrium between the immunosuppression of the immune system and the inoculum load is not always easy to establish. Experimental chronic models of infections mimicking human diseases are undeveloped [10]. Even though such developments may seem scientifically unrewarding, they should be considered to be highly important because an inappropriate experimental animal model may lead to erroneous conclusions regarding fungal virulence factors: two different mouse models of inoculation can identify different sets of low virulent mutants in the same library of mutants (I Mouyna, J Sarfati, JP Latgé, unpublished data). The concept of a virulence factor (VF) in opportunistic fungi and in obligate pathogens (that require an intermediate host to be able to complete their biological cycle) is also different. In the latter, a VF would be a gene or a protein essential for growth in vivo whose deletion does not affect mycelial growth in vitro. In A. fumigatus, for example, auxotrophs that do not grow in vitro are avirulent or cell wall mutants with reduced growth in culture media are less virulent in a mouse model ([11,12]; I Mouyna, J Sarfati, JP Latgé, unpublished data). Is it correct to call a gene whose disruption reduces fungal growth in vitro and consequently affects pathogenicity in an experimental animal model a VF of an opportunistic fungus?

As stated by Lorenz (pp 372–378), our view of fungal pathogenesis will probably change entirely in the near future, following genome analysis of pathogens under study. In addition to the ongoing and almost completed genome projects, it can be hoped that, in the near future, all genomes of human fungal pathogens will be available. Availability of these sequences will, in part, make possible our ability to apply the S. cerevisiae technology to any fungal pathogen. Lorenz presents some very exciting new developments in the genomic analysis of our ‘pet’ fungus,
S. cerevisiae. With the robotization and miniaturization of DNA and protein technologies and the development of powerful biocomputing facilities, it will be possible to integrate classical transcriptome and proteome analysis with data obtained on protein–protein, protein–DNA inter-actions and promoter functions. Such approaches will result in the discovery of entirely new regulatory circuits or networks, especially for the study of a fungus like A. fumigatus (for which >5000 putative ORFs have no homologs in databases), and when scientific manpower is limited (~1% of the S. cerevisiae community).

An integrated genomic approach will, without doubt, greatly help our understanding of fungal morphogenesis and the multigenic nature of virulence in invasive, opportunistic infections. Disruption of genes encoding global regulators of fungal growth in vivo may indeed lead to an avirulent strain, but this may be difficult to obtain, taking into account compensatory genes within or outside gene families or redundancy between pathways associated with the saprophytic life of these fungi. Saprophytic fungi must have developed a sophisticated armamentarium that allows them to survive under different environmental conditions and resist external aggressors that a genome analysis will without doubt identify. In opportunistic bacterial pathogens, such as Pseudomonas aeruginosa, environmental adaptability is associated with a multiplicity of regulatory genes and a high number of genes involved in catabolism, transport and efflux of metabolites and chemosensing systems that allow the bacterium to modulate its biochemical capability in changing environmental conditions [13].

## Antifungal therapy

Therapeutic treatment of invasive fungal infection remains a difficult task, especially amongst immunocompromised patients. There are mainly two reasons for this. First, diagnosis of invasive infections is most often too late and occurs at a time when the fungal burden is too high for an efficient antifungal therapy. Diagnosis of these individuals remains difficult because of the lack of a specific antibody response during immunosuppression. Currently, most diagnostic tools are based on the detection in vivo of molecules that have been identified in vitro (often because they are present in high amounts). For example, serological diagnosis of IA is based on the identification of circulating galactomannan or circulating DNA [14,15]. How these molecules are secreted in vivo is totally unknown. The development of mass spectrometry systems and transcriptome analysis should lead to the identification of fungal molecules that are released at the earliest time of infection or host molecules that are specifically produced in response to the establishment of the fungus in its host.

Second, the number of efficacious drugs available clinically is very limited. Most of them are azoles that inhibit ergosterol biosynthesis. Because of their widespread use, resistant strains have increased in frequency. As shown by Sanglard (pp 379–385), resistance to azoles is due to several mechanisms that vary from species to species. It seems, however, that resistance to azoles, at least in yeasts, is mainly associated with the upregulation of ABC (ATP-binding cassette) transporters or major facilitator (MF) proteins. An analysis of drug resistance mechanisms will also increase our understanding of fungal pathogenicity, as the same proteins mentioned above that cause drug efflux also remove metabolic inhibitors that could be, for example, associated with the oxidative stress response that is an essential component of the innate defense reaction of the host. In addition, overexpression or deletion of the genes encoding multidrug transporters is associated with a variation in the pathogenicity of the strains [16]. Although the efflux of drugs is well understood, the mechanism by which biomolecules penetrate the cell wall and plasma membrane remains obscure. Cell wall permeability is an essential area of research, especially as it is now obvious that the cell wall is not a static but a highly dynamic structure that rearranges itself continuously after environmental changes. The last point mentioned by Sanglard on the difference of drug susceptibility of biofilms as compared to planktonic cells emphasizes data from other studies published on antigenic variation or switching [17,18]. A fungal colony must not be considered to be a single entity, but a heterogenous population of cells with different biological properties. Terminology such as ‘biofilms’ or ‘quorum sensing’, well-known for the bacterial pathogens, is now being applied to the fungal pathogens [19,20]. Focussing on these areas of research should increase our understanding of survival of the fungus in the immunocompromised patient as well as define why antifungal therapies fail.

New alternatives to treating mycoses are also required to circumvent the often poorly efficient (but expensive) fungal treatments. Although new to studies of medically important fungi, data supporting the utility of vaccination, passive antibody prophylaxis or immune reconstitution is appearing with rapid frequency. Human trials using antibody-mediated immunity are ongoing against C. albicans and C. neoformans infections. Other efforts favour the elicitation of a protective cellular immune response. Humoral immunity and vaccines are reviewed by Casadevall, Feldmesser and Pirofski (pp 386–391). Antibody protection is associated with an increased efficiency of cellular immune mechanisms. Currently, the contribution of each component to the total immune response (B and T cells, and phagocytes) in the protective response has not been totally deciphered. One exciting discovery is the identification of good and bad antibodies that either protect or harm patients. Another challenge in vaccination against fungi is the protection of immunocompromised patients at risk. Recent data from our laboratory showed that immunosuppression is not an obstacle to immunoprotection. Mice can be protected against an A. fumigatus infection even after being treated with glucocorticoids that usually trigger experimental IA.

The challenge of the next several years remains to link the biological and ecological properties of the fungus with
the immunosuppressive status of the host in order to understand virulence. There is no doubt that genomic and post-genomic approaches will help our understanding of the multigenic character of virulence. Recent studies have emphasized putative interesting functions of Candida or Aspergillus genes that do not have homologs in the S. cerevisiae genome. In addition, molecular studies have shown that orthologous genes do not display the same function in Candida or Aspergillus and S. cerevisiae, as the phenotype of the respective mutants is very different. Genomic approaches should not be restricted to the fungal pathogens but be extended to include humans. Human genetics may explain the genetic predisposition of the patients to a disease [21]. A classical example of this is a mutation in the NADPH oxidase pathway that is responsible for chronic granulomatous disease always associated with the development of IA [22]. Mannose-binding protein gene polymorphism has also been recognized as a susceptibility factor for chronic necrotizing pulmonary aspergillosis [23].

References