The pathobiology of *Aspergillus fumigatus*

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Aspergillus fumigatus **is the most prevalent airborne fungal pathogen in developed countries, and in immunocompromised patients causes a usually fatal invasive aspergillosis (IA). Understanding the pathobiology of this fungal species requires not only analysis of the putative fungal virulence factors that stimulate fungal growth and/or survival in the lung environment, but also knowledge of the immune factors containing** ***A. fumigatus*** **in the immunocompetent host that can be debilitated by immunosuppressive therapies, triggering IA. Although the incidence of IA has dramatically increased in recent years, progress in these areas has been limited and, as yet, a single, true virulence factor has not been identified and the mechanisms responsible for protective immunity against** ***A. fumigatus*** **have yet to be elucidated.**

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The saprophytic species *Aspergillus fumigatus* is found worldwide and has an essential role in recycling carbon and nitrogen. This fungal species has a very simple biological cycle (Fig. 1), one characteristic of which is its high sporulating capacity, which results in the ubiquitous presence of high concentrations of conidia (1–100 conidia m^{-3}) in the air indoors and outdoors. Indeed, *A. fumigatus* conidia are continuously inhaled by humans but rarely have any adverse effects as they are eliminated efficiently by the innate immune response. However, the harmless status of *A. fumigatus* has changed over the past 10–20 years, owing to the increasing number of immunosuppressed patients and an increase in the severity of immunosuppressive therapies. *A. fumigatus* is now the most prevalent airborne fungal pathogen, and causes severe and usually fatal invasive infections in immunocompromised hosts in developed countries[1,2].

In spite of a dramatic increase in the incidence of invasive aspergillosis (IA), the pathogenicity of *A. fumigatus* is still poorly understood. Because of the unique nature of the infection – a saprophytic fungus colonizing an immunocompromised host – understanding its molecular basis requires consideration of the host defence reactions that can contain *A. fumigatus* in an immunocompetent host but that can be inhibited by immunosuppressive therapies.

Analysis of fungal aggressiveness

The airborne conidia of *A. fumigatus* have a diameter small enough (2–3 m) to reach the alveoli in the lung. If the conidia can overcome the immune defence mechanisms in the lung, they germinate and produce a branched, septate vegetative mycelium that invades the lung tissues. The virulence of *A. fumigatus* can then be caused either by the production of fungal proteins that promote mycelial growth into the lung parenchyma or by structural features of the conidia that confer resistance to the host's antifungal mechanisms. Different molecular strategies have been developed to explore these two areas of research.

Molecular strategies to investigate A. fumigatus *virulence*

To date, the study of *A. fumigatus* virulence has been based on the analysis of mutants constructed by gene disruption, and the detection of attenuated virulence consistent with the inactivation of a gene encoding a virulence factor. The classical method of constructing *A. fumigatus* mutants involves disrupting the gene of interest by inserting an antibiotic-resistance gene. Only two genes, one conferring resistance to hygromycin and the other to phleomycin, have been used so far[3,4]. The *URA* blaster method previously developed in yeast has been adapted to *A. fumigatus*, in which it is known as the *PYRG* blaster[5,6]. This system should allow multiple successive mutations. Recently, transposon mutagenesis (using the *Agrobacterium* Ti plasmid or class I and class II fungal transposons) has also been used to construct *A. fumigatus* mutants[7]. If UV or chemical mutagenesis produces a mutant with an interesting phenotype, the mutated gene can be identified by complementation using a genomic library to recover the wild-type phenotype[8,9].

Two strategies have been used to investigate *A. fumigatus* virulence using mutants (Fig. 2). In the first approach, putative virulence factors are selected in advance, based on the biochemical function of the protein (e.g. protease, oxidase, phospholipase or toxin), which, potentially, could help the invading mycelium to grow *in vivo*. The main drawback of this analysis is that the identification of the function of the protein is based on a fungus grown *in vitro*. The second approach is the construction of libraries of mutants by random insertional mutagenesis. Conditions for restriction-enzyme-mediated integration (REMI) have recently been published[10]; *Xho*I and *Kpn*I digestion favours single-copy integration of transforming DNA in the majority of transformants. The signature-tagged mutagenesis (STM) approach developed for bacteria has also been applied to *A. fumigatus*[11].

Animal models play a central role in identifying virulence factors (Fig. 2). Although mice are the most commonly used test animals, there is no consensus on the best experimental model to use (for a review of animal models, see Ref. 1). The identification of virulence factors does, however, depend on the experimental model used[12]. Variations in the model

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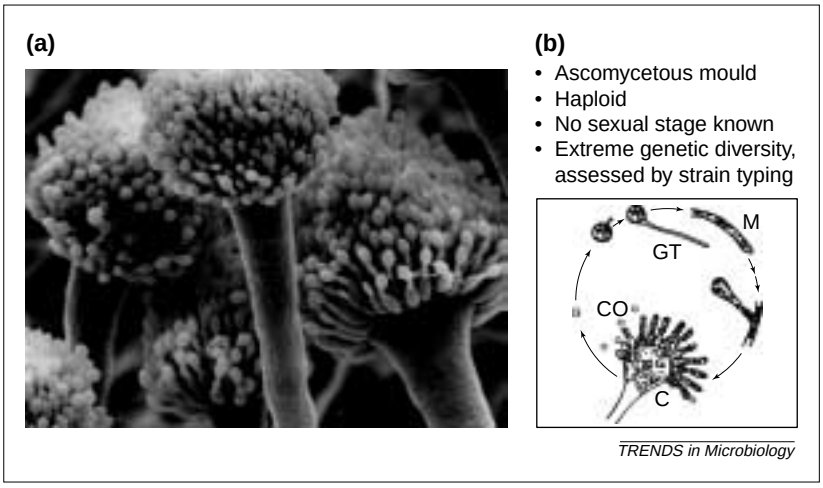

Fig. 1. (a) *Aspergillus fumigatus*. *A. fumigatus* grows in organic debris. Conidia are released into the air, inhaled by humans and cause severe invasive aspergillosis (IA) in immunocompromised patients. (b) The microscopic characteristics of *A. fumigatus*. C, conidiophore with conidia produced in basipetal succession; CO, conidia; GT, germinating conidia; M, vegetative mycelium.

concern the choice of animal (strain, weight and sex), the immunosuppressive regimen (drugs, dose and the frequency of the injection) and the infection protocol (concentration of conidia and the route of injection). Three major issues must be considered regarding the animal experiments:

(1) Immunosuppressive treatments substantially increase the susceptibility of animals to infection. As in human infections, the more severe the immunosuppressive treatment, the more susceptible the animals are to IA. Because they are easier to use, cortisone and/or cyclophosphamide are preferred for immunosuppression in animals[13,14].

(2) Intranasal inoculation mimics the natural route of infection and seems to be a more appropriate route than intravenous (i.v.) injection. As in humans, following inhalation of conidia (and in contrast to i.v. injections), the lung is the primary target organ and disease only develops if the animal is immunosuppressed; an immunocompetent mouse can clear as many as 10^8 conidia without developing disease[1].

(3) In the usual approach, strains of *A. fumigatus* are inoculated into separate groups of animals and death is monitored over time. This method is only appropriate for identifying mutant strains characterized by large differences in virulence. Models involving mixtures of strains are more useful to quantify slight variations in strain aggressiveness that are not detected when isolates are inoculated individually[15]. Coinfection of a mutant and its parental strain has been used to demonstrate reduced virulence of a glucanosyltransferase mutant with reduced growth rate (I. Mouyna *et al*., unpublished). Infection with pools of several strains has allowed the screening of 96 STM-generated mutants per mouse[11].

The major drawback of animal models is the lack of conformity to human infection and, although it is urgently required, there is no animal model in which IA is induced by a low concentration of conidia. Experimentally induced aspergillosis is a hyperacute infection obtained by administering a high concentration of conidia at a single time point. In humans, IA is often a more chronic disease, caused by continuous or repeated exposure to small numbers of conidia. The inoculum size can be reduced if the severity of the immunosuppression is increased. The drawback to this approach is the susceptibility of the immunocompromised animals to other infections. One promising method is the use of transgenic mice such as interferon (IFN)-$\gamma^{-/-}$, interleukin (IL)-$12^{-/-}$ or X-linked chronic granulomatous disease (X-CGD) (NADPH oxidase-minus) mice that are more suceptible to infection; for example, X-CGD mice can be infected with an inoculum of 50 conidia per mouse[16–18].

Proteins secreted during mycelial growth putatively involved in virulence

Table 1 lists the enzymes and toxins secreted by *A. fumigatus* that have been analyzed genetically.

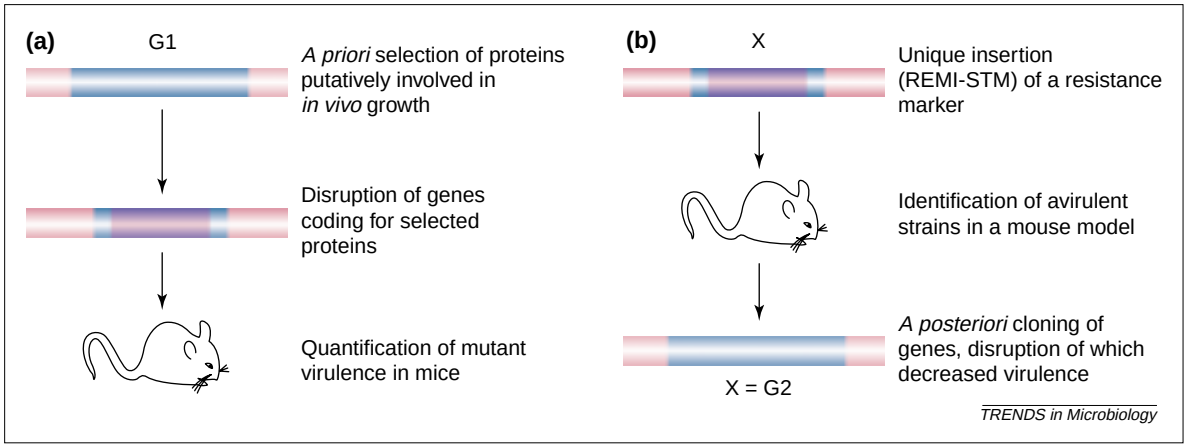

Fig. 2. Strategies to identify genes (G1 and G2) encoding putative virulence factors of *Aspergillus fumigatus*. (a) The gene (G1) encoding a protein with an enzymatic activity that should favour the growth of *A. fumigatus* in the lung (e.g. a protease) is disrupted; the mutant obtained is tested in an experimental model of murine aspergillosis, and the survival of mice inoculated with the mutant is compared with the parental strain. (b) A library of mutant is constructed by random mutagenesis ; pools of mutants (each one carrying a differential tag) are inoculated together into a single mouse; mutants that cannot be recovered from the lung carry a disruption in a gene (G2) essential for pathogenicity that can then be cloned. Abbreviations: REMI, restriction-enzyme-mediated integration; STM, signature-tagged mutagenesis.

Table 1. *A. fumigatus* molecules produced during mycelial growth with a putative role in virulence

Category	Molecule	Role *in vivo*	Mutant available
Toxic molecules	Ribonuclease	Host cell death	Yes
	Haemolysin	Red blood cell lysis	No
	Secondary metabolites e.g. gliotoxin	Immunosuppression	No
Enzymes	Serine protease,	Promote lung matrix	Yes
	Aspartic protease,	colonization and/or	Yes
	Metalloprotease,	degrade humoral	Yes
	Dipeptidylpeptidases	factors	No
	Catalases,	Antioxidants during	Yes
	Superoxide dismutases	phagocytosis	No
	Phospholipases	Epithelial damage	No

None of these factors has been shown to be involved in the pathogenesis of *A. fumigatus* in experimentally induced infections[4,13,14,19,20]. The number of proteins analyzed remains very low, and toxins, such as secondary metabolites or haemolysin, and enzymes such as phospholipases or superoxide dismutase, have not yet been analyzed at the molecular level[21–24]. The analysis of randomly selected mutants has been just as disappointing. Among 5000 REMI–STM mutants tested in the lung environment, the only avirulent mutant identified to date is a p-aminobenzoic acid auxotroph that is not able to germinate *in vivo* when this metabolite is not freely available[11]. Similar attenuation was previously observed for a *pyrG* mutant unable to germinate *in vivo* in the absence of uridine and uracil[25]. The term virulence factor, however, should not be applied to such factors but reserved for factors that, when mutated, do not affect morphogenesis *in vitro*.

DNA-fingerprinting studies have confirmed these experimental data, and shown that it is impossible to discriminate between clinical and environmental isolates of *A. fumigatus*, which also indicates that every strain present in the environment is potentially pathogenic if it encounters the appropriate host[26,27]. Indeed, IA can easily be induced in animal models using a strain isolated from the environment or from a different host, providing evidence for the absence of host specificity in *A. fumigatus* pathogenesis. In addition, and in contrast to most other pathogenic microorganisms, completely avirulent strains of *A. fumigatus* have not been reported, making complementation studies for the identification of virulence factors impossible.

All the data accumulated to date suggest that the mycelial virulence of *A. fumigatus* is multifactorial. If this hypothesis is valid, a strategy based on single-gene deletions is inappropriate to study the virulence of this fungus, unless a regulatory gene controlling a cluster of genes involved in pathogenesis is identified. One example of this multifactorial interaction with the host is demonstrated by the capacity of *A. fumigatus* to adhere to a set of host proteins (laminin, fibrinogen, surfactant A and D, mannose-binding lectin, complement, immunoglobulin and fibronectin) through a variety of different cell-wall receptors. Only large-scale transcription studies will allow the identification of clusters of genes associated with the mycelial growth of *A. fumigatus in vivo.*

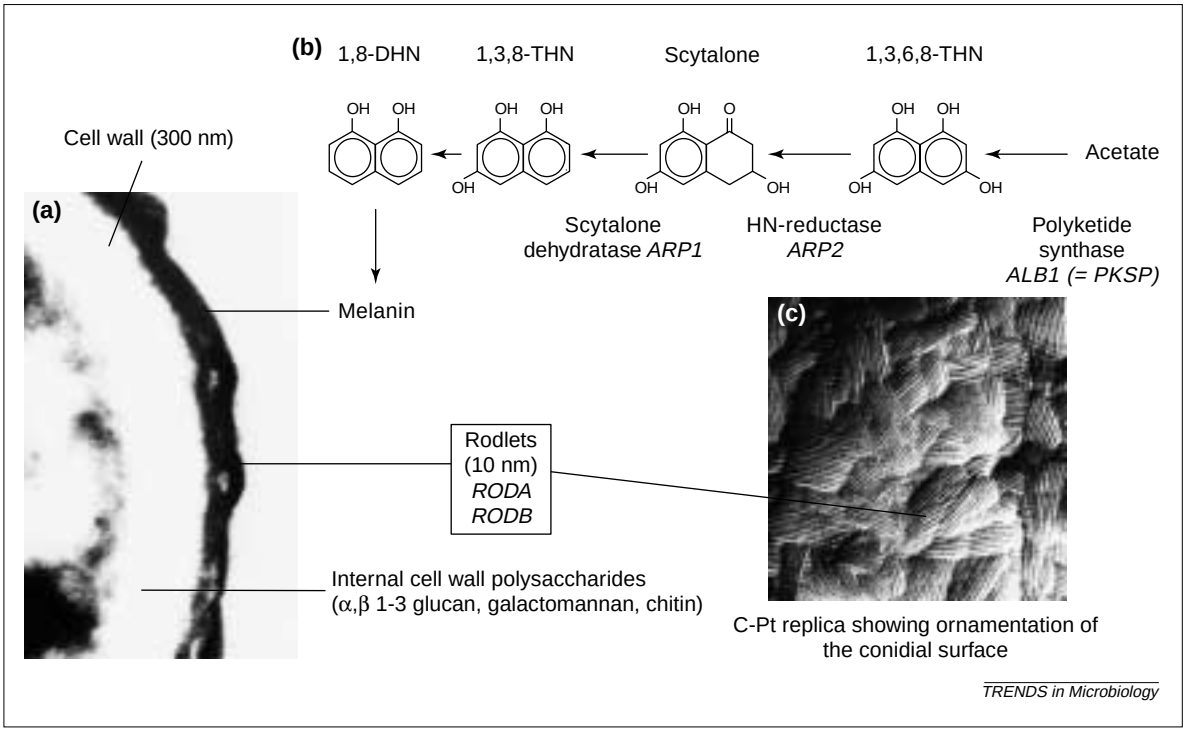

Fig. 3. Structural organization of the conidial cell wall of *Aspergillus fumigatus.* (a) An electron microscopy section of the conidial cell wall. The translucent inner layer contains structural polysaccharides and proteins whereas the electron-dense outer layer contains the melanin. (b) The major steps in the melanin biosynthetic pathway and their associated genes. (c) The structural arrangement of the layer of the hydrophobic proteins encoded by *RODA* on the conidial surface. Abbreviations: DHN, dihydroxynapthalene; THN, tetrahydroxynapthalene.

Constitutive cell-wall molecules involved in A. fumigatus *resistance to phagocytosis*

The conidia of *A. fumigatus* are covered by a layer of hydrophobic proteins that can be seen after unidirectional metal shadowing under electron microscopy as a regularly arranged surface layer, known in mycology as the 'rodlet layer' (Fig. 3). The regular arrangement of these surface proteins confers hydrophobic properties to *A. fumigatus* conidia. The gene (*RODA*) encoding the protein responsible for the rodlet structure has been cloned and a rodlet-less mutant with hydrophilic conidia has been generated[3]. Conidia from this mutant do not bind readily to proteins with hydrophobic pockets, such as albumin or collagen, but do bind to other host proteins such as laminin and fibrinogen. In an animal model of pulmonary IA, mortality was comparable for the parent and rodlet-less strains but the inflammatory response was lower with a Δ*rodA* mutant[12]. Thus, RodAp might play a role in the hydrophobic interactions between fungus and host, but these interactions do not appear to be essential for fungal virulence. A second hydrophobin gene (*RODB*) has recently been identified in *A. fumigatus* (S. Paris *et al*., unpublished).

A dense, pigmented outer layer is adjacent to the rodlet layer (Fig. 3). The conidial pigment is synthesized through the dihydroxynaphthalene (DHN)–melanin pathway[8,9,28–30]. To date, molecular studies have identified six genes involved in the DHN–melanin biosynthetic pathway (Fig. 3). The six genes form a cluster spanning 19 kb and are all developmentally regulated and expressed during conidiation. *ALB1* (*PKSP*) encodes a putative naphthopyrone synthase and is homologous to *WA* (white A) in *Aspergillus nidulans*. *ARP1* and *ARP2* encode a scytalone dehydratase and a 1, 3, 6, 8 tetrahydroxynaphthalene reductase, respectively. *ABR1* and *ABR2* have signature sequences characteristic of oxidases: a multicopper oxidase (*ABR1*) and a laccase (*ABR2*) homologous to *YA* (yellow A) of *A. nidulans*. The function of the sixth gene (*AYG1*) remains unknown. Analysis of mutants with white conidia resulting from the inactivation of *ALB1* has shown that the melanin layer of the cell wall plays a major role in protection against the phagocytic reactions and that white conidia are damaged more efficiently by phagocytes than wild-type conidia are. *In vitro* and *in vivo* assays suggest that the greater survival of wild-type conidia correlates with efficient scavenging of reactive oxygen species by the conidial pigment[31]. Very similar results have been obtained with *Wangiella dermatitidis* and *Cryptococcus neoformans*, where the ability to scavenge reactive oxidants has been linked to the presence of the pigment. Mutations in the melanin pathway also result in modification and/or reorganization of the cell wall – white conidia have a smooth surface whereas wild-type conidia have distinct ornamentation on their surface[32]. The chemical modifications associated with the change in surface ornamentation remain unknown; however, surface receptors must be modified as a significant elevation in the binding capacity for complement component C3 and quicker engulfment by alveolar macrophages are seen in mutant conidia[8].

The reduction in fungal pathogenicity caused by inhibition of the melanin pathway remains limited, however. In an i.v. murine infection model, the virulence of white conidia was reduced by approximately 20–50% compared with wild type[32] and non-pigmented conidia can still induce IA when inoculated intranasally into steroid-treated mice. Nevertheless, in a study of mixed infections with wild-type and mutant strains of *A. fumigatus*, strains with white conidia were recovered in lower amounts than strains with green conidia, confirming they are less virulent than wild-type green strains, even in an immunocompromised experimental murine model[15]. However, the role of melanin in virulence has no implications for the control of IA, as >99.99% of airborne conidia are green and their invading mycelia are not pigmented.

Host defence mechanisms counteracting *A. fumigatus* growth in the lung

In the immunocompetent host, *A. fumigatus* is seldom pathogenic because it is killed by the innate immune system. It is the downregulation of the immune system caused by immunosuppressive therapies and congenital defects that triggers the development of IA in experimental models as well as in naturally occurring human infections. Under these conditions, the biological characteristics of the fungus (a fast-growing thermophillic species with conidia of small diameter and no specific nutritional requirements) could be sufficient for virulence. The major cellular innate defence mechanisms that the fungus must overcome are shown in Fig. 4.

Lung epithelium

The majority of *A. fumigatus* conidia, like most aerosol particles, are excluded from the lung by the ciliary action of the mucous epithelium. Although the proportion of conidia remaining in the respiratory tract after inhalation has not been quantified, the clearance of *A. fumigatus* at this level might be less efficient than with other airborne saprophytes, as *A. fumigatus* toxins have been shown to inhibit ciliary activity, and proteases can damage the epithelial tissue[33,34]. In addition, epithelial and endothelial cells have been shown to internalize conidia, serving as putative foci of infection[35]. In animal models, penetration at the epithelial level is a common event. An area of research that has not been explored to date is the role of damage to the lung epithelium following immunosuppressive therapies (irradiation and drugs) and in graft-versus-host disease[36], as the binding of conidia to epithelial cells is facilitated if epithelial cells are altered or activated. In addition, the role of antimicrobial peptides and proteins constitutively

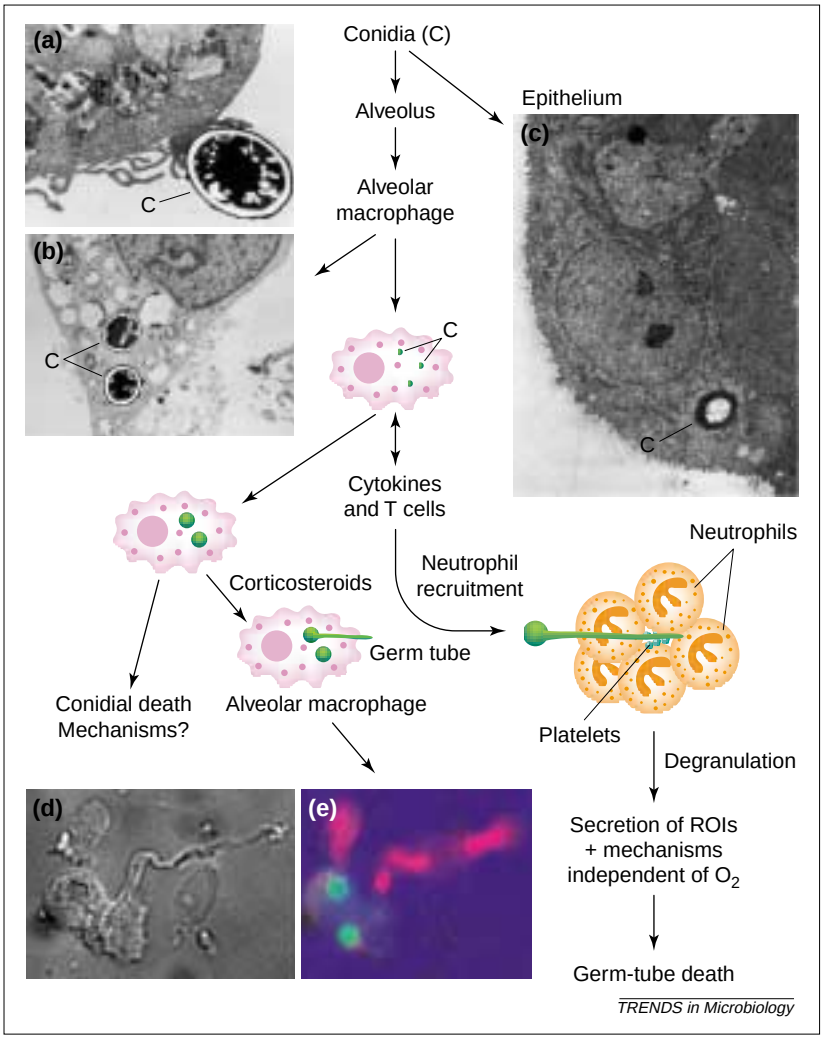

Fig. 4. Successive phagocytic events occurring after *Aspergillus fumigatus* conidia invade the lung. (a) Conidia (C) adhere to and are internalized either by alveolar macrophages (b) or by an epithelial cell (c). In the absence of immunosuppression, conidia are killed; in the presence of glucocorticoids, conidia germinate inside the alveolar macrophages and kill them. (d) and (e) show conidia germinating in an alveolar macrophage of a corticone-acetate-treated mouse. The conidia are FITC-labelled before phagocytosis and germ tubes are labelled post-phagocytosis with an anti-conidium antibody and a rhodamine anti-rabbit Ig conjugate. The region of the germ tube inside the macrophage is not labelled whereas the germ tube extruding from the macrophage is stained red. Germ-tube growth inside the alveolus induces neutrophil recruitment; hyphae are susceptible to killing by neutrophils following degranulation. Abbreviations: ROI, reactive oxygen intermediate.

synthesized at epithelial surfaces in controlling *A. fumigatus*[37–39] has not yet been investigated. Interestingly, high salt concentration and corticosteroids (a situation common in cystic fibrosis patients or transplant patients at risk for aspergillosis) reduce the efficiency or the global release of these compounds[37,38].

Interaction of conidia with alveolar macrophages

The recognition of conidia by alveolar macrophages has been poorly studied, although lectin-like interactions are thought to be primarily responsible for adherence and ingestion of conidia. The mannosyl-fucosyl receptor and two other receptors (inhibited by β glucan and chitooligosaccharides) have been suggested to mediate conidial binding, but specific receptors have not been identified[40,41].

After phagocytosis, the first stage of germination, namely conidial swelling, is not inhibited, even in immunocompetent alveolar macrophages. Killing of conidia starts 6–8 h after phagocytosis, a delay corresponding to germ-tube emission *in vitro*. The killing rate is surprisingly slow, with only a 10% or less reduction in conidia viability after 6 h of phagocytosis. These data correlate well with the slow elimination rate of conidia from the lungs of mice following a respiratory challenge (2–3 days, depending on the initial inoculum load) (B. Philippe *et al*., unpublished). The antimicrobial system(s) responsible for killing conidia have not been identified, and data on the role of reactive oxygen intermediates and nitric oxide are contradictory[42–47]. Cell biology studies have recently shown that conidia of *A. fumigatus* are rapidly internalized into a phagolysosome (O. Ibrahim-Granet *et al*., unpublished), but the role of lysosomal enzymes in conidial killing is not understood. In addition, the presence of chitinases in the lung and the increase in the serum level of chitinases, especially during *Aspergillus* infection[48,49], suggest that glycosylhydrolases could play a significant role in the degradation of the inner layer of the conidial cell wall.

Interaction of mycelia with neutrophils

Polymorphonuclear neutrophils (PMN) are recruited to the lung when the fungal burden is high, especially following extracellular fungal germination. Neutrophils adhere to the surface of the hyphae, as hyphae are too large to be engulfed. The mechanisms of adhesion and the fungal and PMN receptors involved in these interactions remain to be elucidated.

Contact between neutrophils and hyphae triggers a respiratory burst, secretion of reactive oxygen intermediates and degranulation[50–55]. In contrast to the killing of conidia by macrophages, hyphal damage by PMN is rapid, in that 50% of the hyphae were killed after a 2 h incubation. Killing of hyphae requires oxidants, but the release of oxidants from PMN could not mediate hyphal killing without concomitant fungal damage by other components of the enzymatic granules. PMN-induced cell-wall damage was detectable before killing occurred, and incubation of hyphae with granules isolated from PMN resulted in a very rapid release (<30 min) of cell-wall glycoproteins (R. Diamond, unpublished). The enzymes responsible for damage to the hyphal cell wall have not been identified. Cationic peptides, such as the defensins, might also be active against germinating conidia[43].

Modification of the immune response modifies host sensitivity to A. fumigatus

Among the drugs used in the management of patients at risk for IA, corticosteroids play a major role in disease progression[56,57]. The specific role that corticoids play in the interaction of *A. fumigatus* with the immune systems is poorly understood[58,59]. Glucocorticoids have pleiotropic immunosuppressive

Questions for future research

- *A. fumigatus* is the species responsible for >90% of IA in humans. Is IA caused by virulence factors specific to *A. fumigatus* or by the ubiquitous occurrence of the thermophilic *A. fumigatus* in the atmosphere?
- Lower virulence of *A. fumigatus* has always been associated with reduced growth rate. Will studies on signal transduction cascades, hyphal elongation and growth polarization allow a greater understanding of the development of *A. fumigatus* infection *in vivo* than the search for true virulence factors?
- How can large-scale genomic studies help our understanding of *A. fumigatus* virulence? For example, will comparative genome analysis of the pathogenic *A. fumigatus* and non-pathogenic *A. nidulans* identify *A. fumigatus* virulence traits?
- Glucocorticoids are the key trigger in the establishment of IA. What are the host innate immune mechanisms essential for the killing of *A. fumigatus* that are inhibited by glucocorticoid treatment?
- HLA-DR2 and DR5 molecules contribute to susceptibility of patients to allergic broncho-pulmonary aspergillosis. What are the host genetic factors that contribute to IA susceptibility?
- A role for the T cell in protective immunity has been demonstrated in experimental animal aspergillosis. How specific is the T-cell response and is vaccination a prophylaxis of the future for IA?
- Will the use of transgenic mice be the best method to develop an animal model of chronic IA and to better understand the specificity of the host immune response against *A. fumigatus*?

effects; among the effects that could promote *A. fumigatus* development are the promotion of a Th2-cytokine-secreting profile and a decrease in the production of reactive oxidants and nitric oxide[60,61].

In recent years, it has been shown that T-cell immunity and associated cytokines and/or chemokines have a role in the control of *A. fumigatus* infection. The development of protective immunity against *A. fumigatus* is associated with the production of the Th1 cytokines IL-18, tumour necrosis factor (TNF)-α, IFN-γ and IL-12; IL-18 is the first of this cytokine network to be detected[62–66]. Accordingly, *ex vivo*, granulocyte-stimulating factors and IFN-γ have been shown to stimulate the phagocytic mechanisms, and are able to reverse the corticosteroid effect[67,68]. By contrast, it has been shown that progression of IA is associated with a $CD4^+$ Th2 response, which correlates with an increase in IL-4 and IL-10 levels and a decrease in the levels of IFN-γ (Refs 16,17,65,69). The mechanisms underlying the activation of Th1 or Th2 cells by *A. fumigatus* have not yet been defined. In addition, chemotactic cytokines involved in the recruitment and activation of mononuclear and PMNs have recently been shown to play a role in the immune response against *A. fumigatus*[70,71].

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Concluding remarks

The main difficulty in studying the virulence of the human opportunistic fungal pathogen *A. fumigatus* lies in its lack of pathogenicity towards the immunocompetent host. Invasive pulmonary infections are mostly seen in immunocompromised hosts, in conjunction with the inhibition of the immune-defence mechanisms that normally clear *A. fumigatus* infections. Accordingly, molecular studies have been unable to identify a single, true virulence factor essential for the development of *A. fumigatus* in the lung. It is thus tempting to speculate that *A. fumigatus* virulence is directly correlated with its specific biological characteristics: the small size of the conidia allows their dispersal throughout the entire respiratory tract; mycelial growth at temperatures >37°C is rapid; and it has no specific nutritional requirements. However, the fact that *A. fumigatus* is responsible for >90% of human pulmonary mycosis suggests that this fungal species does indeed have some molecular features that favour its development in human tissues. *A. fumigatus* virulence must thus be polygenic, and the only way to identify global regulators or multiple genes expressed concomitantly is by transcriptome and/or proteome analysis of the fungus grown *in vivo*. Such an approach will be possible when the entire genome sequence of *A. fumigatus* becomes available (a genome sequencing project directed by an international consortium led by TIGR and the Sanger Centre was launched this year; for progress see http://www.aspergillus.man.ac.uk).

One of the key events in the establishment of aspergillosis is the resistance of *A. fumigatus* to phagocytosis and its slow killing *in vivo*. Moreover, even though alveolar macrophages and neutrophils have been shown repeatedly to play an essential role in the killing of *A. fumigatus* conidia and germ tubes, respectively, the molecular mechanisms responsible for the killing of the conidia and mycelia remain to be elucidated. Similarly, studies of crosstalk between T cells and phagocytes remain limited to an analysis of Th1/Th2 cytokine patterns in murine aspergillosis without considering human IA infections. These are key research areas to develop if we want to improve protective immunity against this fungal infection.

This review shows the limitations of our knowledge of the pathobiology of *A. fumigatus*. Future studies are needed to further our understanding of how this fungal disease becomes established and how it develops, an essential requirement for the better management of patients with IA, the number of which will most certainly increase in the near future.

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Campylobacter jejuni – microtubule-dependent invasion

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Campylobacter jejuni **is the leading bacterial cause of food-borne illness worldwide and a major cause of Guillain–Barré paralysis. Recent molecular and cellular studies of one well-characterized** ***C. jejuni*** **strain have begun to unravel the details of an unusual microtubule-dependent (actin-filament-independent) gut-invasion mechanism, through which at least some** ***C. jejuni*** **initiate disease. Although responsible for causing a human dysenteric syndrome remarkably similar to that triggered by** ***Shigella*** **spp., current evidence suggests that** ***C. jejuni*** **use some markedly different molecular mechanisms of pathogenesis compared with shigellae.**

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Bacterial dysentery is an acute, invasive, inflammatory infection of the terminal ileum and colon, which can result in diarrhea or classical dysenteric syndrome (i.e. fever, tenesmus, malaise, myalgia, headache, severe abdominal cramps and frequent, small-volume rectal discharges of mucus, inflammatory cells and necrotic tissue containing streaks of blood). For most of the 20th century, this human infectious disease was thought to be caused primarily by members of the genus *Shigella* and, infrequently, by the related enteroinvasive subgroup of *Escherichia coli* (EIEC), which shares its pathogenic mechanism with *Shigella*. However, studies conducted over the past 20 years have clearly shown that *Campylobacter jejuni* also causes dysentery. Although often undiagnosed owing to its strict microaerophilic nature, *C. jejuni* is now recognized as the leading bacterial cause of food-borne disease in the USA and many other parts of the world[1,2]. Additionally, enteritis caused by specific *C. jejuni* serotypes is an important precondition for Guillain–Barré paralysis[3].

Bacterial pathogens have evolved mechanisms to resist normal host defenses and subvert host cell processes to initiate disease[4,5]. Invasive bacterial pathogens are now known to interact with the host via intimate biochemical crosstalk, stimulating signaling cascades in both the bacterium and the host that ultimately trigger rearrangements of the host cytoskeleton and cause internalization of the pathogen. The cytoskeleton of eukaryotic cells is a complex array of proteins organized into filaments and accessory proteins. The most prominent filaments are actin filaments and microtubules (MTs), which comprise dynamic strands of actin or tubulin monomers, respectively. These filamentous structures, together with intermediate filaments, are involved in both cellular and subcellular movements, in the determination and maintenance of the shape of host cells and, not surprisingly, in bacterial invasion. Until about 10 years ago, most bacterial invasion pathways were thought to have an exclusive requirement for host actin filaments (i.e. as observed for *Shigella, Yersinia* and *Salmonella*). Since then, the internalization of some members of several bacterial genera, including *C. jejuni*, into host cells has been reported to have a strong requirement for MTs (reviewed in Ref. 6).

The following sections are aimed at summarizing: (1) the known molecular mechanisms of pathogenesis of bacterial dysentery; (2) our current understanding of the cell biology of a novel, MT-dependent mechanism for *C. jejuni* invasion of the intestine; and (3) some important limitations to and current gaps in our understanding. Note that the term 'invasion' is used here to describe the process of bacterial inducement of uptake into non-phagocytic host cells, a specific virulence trait of invasive bacterial pathogens such as *Shigella* or *Yersinia*. Bacterial invasion should not be confused with the medical

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