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Aspergillus fumigatus, a saprotrophic pathogenic fungus

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Aspergillus fumigatus is the most common and life-threatening aerial fungal pathogen, especially among immunocompromised hosts. Understanding the pathobiology of this pathogen requires investigations both to characterise the immunosuppressive status of the phagocytic cells that usually kill the fungus in the immunocompetent host, and to identify fungal virulence factors that either promote the resistance of conidia to innate immunity or stimulate mycelial growth in the lung parenchyma. Recent findings are presented in this review and show how little we know of the interactions between host and pathogen. Now the genome of *A. fumigatus* has been sequenced, transcriptome/ proteome-based studies should disclose the multiple facets of *A. fumigatus* pathogenicity.

Keywords: *Aspergillus*, aspergillosis, virulence, innate immunity

Aspergillus fumigatus is a worldwide saprotrophic species that plays an essential role in recycling carbon and nitrogen. This fungus has a very simple biological cycle (Fig 1), with a high sporulating capacity which results in the ubiquitous presence of high concentrations of conidia (1-100 conidia/m^3) in the atmosphere indoors and outdoors (Lacey, 1996). Conidia of *A. fumigatus* are continuously inhaled by humans and are eliminated efficiently by innate immune mechanisms. Human infections, however, have been regularly reported since the mid 1800's and

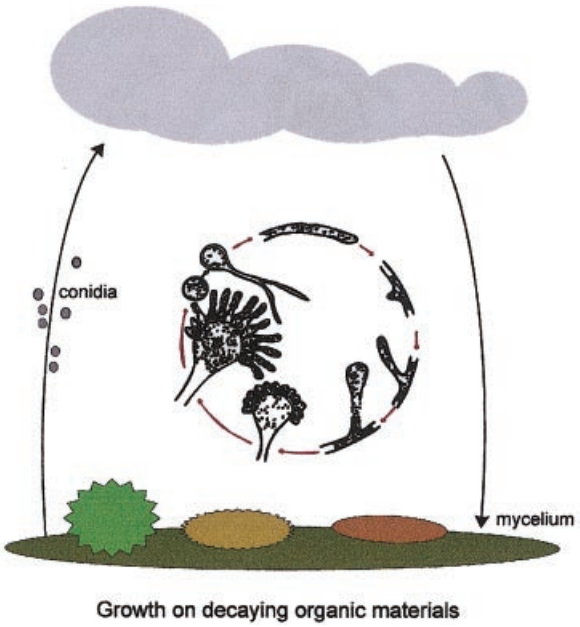

Fig 1 Biological cycle of *A. fumigatus* in nature.

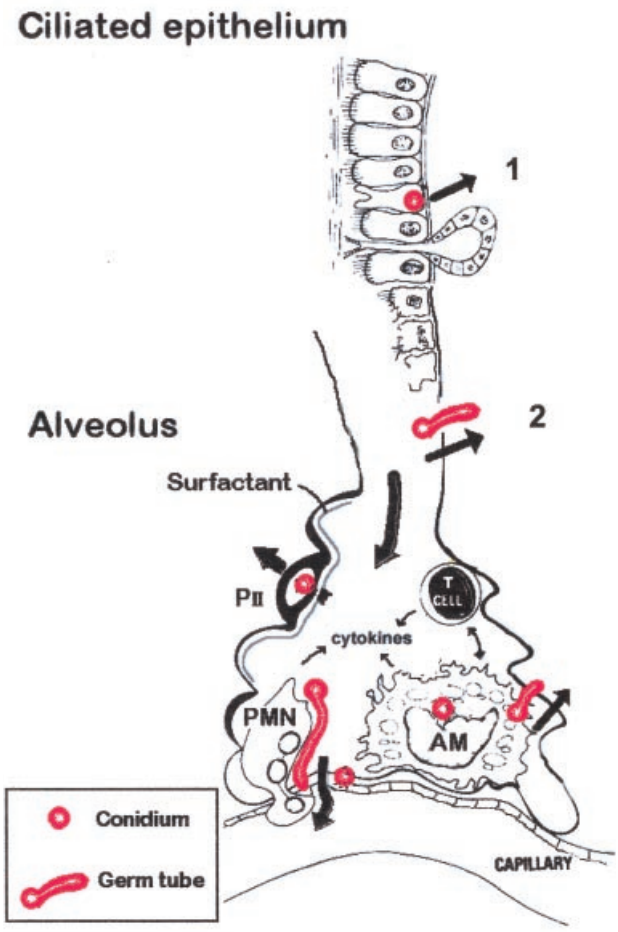

Fig 2 Putative sites of infection for *A. fumigatus*. At the epithelium level, conidia are engulfed (1) or germinating conidia can go through the damaged epithelium (2). In the alveolus, conidia are phagocytosed by alveolar macrophages (AM, 3) or pneumocytes (P II, 4). Following germination due to immunosuppression, germ tubes can escape neutrophils (PMN, 5) and traverse endothelium.

at that time aspergillosis was generally considered a disease of pigeon breeders. Until the 1980s, aspergillosis was considered to be a rare disease, occuring among people with previously formed lung infection cavities caused by diseases such as tuberculosis. The exotic status of *A. fumigatus* has, however, changed in recent years owing to the increase in the number of immuno-suppressed patients and the degree of severity of immuno-suppressive therapies. *A. fumigatus* is now the most prevalent airborne fungal pathogen, and causes severe and usually fatal invasive infections in immuno-compromised hosts in developed countries (Denning, 1998, Latgé, 1999) (Table 1).

Table 1. Pathologies associated with *A. fumigatus* (responsible for 90% of cases of aspergillosis).

Disease	Immune Status	Mycelial growth	Severity
Allergy	IC[(1)]	-	-
Aspergilloma ABPA	IC	+	+
Invasive aspergillosis	IS	+++	+++[(2)]

[(1)] IC: immunocompetent, IS: immunocompromised
[(2)] 50-100% mortality in leukemic/transplant patients infected with *Aspergillus*

More recently, another poltical and environmental issue has arisen concerning *A. fumigatus*. In its natural habitat, this species is found in high concentrations in compost. Surrounding compost plants, conidial concentrations higher than 10^6 conidia/m^3 are often encountered, with similar conidial concentrations often found in material arriving at the composting plant and in the resulting commercial product (Beffa *et al*., 1998 ; Millner *et al*., 1977). This may represent a biological hazard for the general population, as *A. fumigatus* is known to produce polypeptide allergens responsible for asthma and rhinitis, mycotoxins and ß1,3 glucans that are known modulators of the immune system (Heederik *et al*., 2000; Fisher *et al*., 2000; Rylander, 1999, Sigsgeard *et al*., 2000). The allergy risk for the normal population has not been carefully evaluated, especially as symptoms of non-fungal allergy may be exacerbated following exposure to *A. fumigatus*. In the absence of a serious evaluation of the allergic and infectious risks, *A. fumigatus* may represent a biological 'excuse' to prevent the establishment of a composting plant in a selected site.

Apart from this environmental issue, the major problem caused by *A. fumigatus* remains invasive aspergillosis (IA). In spite of a dramatic increase in incidence, the pathobiology of *A. fumigatus* remains poorly understood.

Establishment of the disease

Investigations aiming to discover the initial sites of *A. fumigatus* infection, an essential event in the understanding of IA, have been limited. Fig 2 shows that two putative pulmonary sites of infection can be suspected: the epithelium and the alveola.

Lung epithelium

The majority of conidia of *A. fumigatus*, like most aerolized particles, are excluded from the lung through the ciliary action of the mucous epithelium, although the proportion of conidia not excluded mechanically after inhalation has not been quantified. The role of the epithelium in the establishment of IA has not been carefully analysed yet, although several observations suggest that the epithelium could serve as a focus of infection: (1) epithelial cells can engulf conidia which subsequently remain alive intracellularly (Paris *et al*., 1997; Wasylnka & Moore, 2002) ; (2) the lung epithelium is damaged following immunosuppressive therapies (irradiation and drugs) and graft rejection (Cordonnier *et al*., 1996) facilitating the binding of conidia to altered or activated epithelial cells ; and (3) corticosteroids, a risk factor for IA, reduce the release or efficacy of antimicrobial peptides and proteins constitutively synthesized at epithelial surfaces (Travis *et al*., 1999).

Alveola

Conidia that have not been trapped intracellularly at the epithelial level end up in the alveola where they encounter the main lung phagocytic cell, the alveolar macrophage (AM). Conidial engulfment is very quick (1-2h) and not affected by the immune status of the host. Killing of conidia, mainly due to reactive oxygen intermediates (ROIs), starts 6-8 hours after phagocytosis. The killing rate is surprisingly slow with only a 10% or less reduction in conidia after 6 hours of phagocytosis. These data correlate well with the slow elimination rate of conidia seen in the lungs of mice

following a respiratory challenge (2-3 days depending on the initial inoculum load) (Levitz *et al*., 1986; Philippe *et al*, 2003). Immunosuppression by corticoids is associated with a reduction in ROI production that is correlated with the intramacrophagic germination seen in these patients.

Polymorphonuclear neutrophils (PMN) are recruited to the lung following extracellular fungal germination. They represent the third line of defence against *A. fumigatus*, explaining why neutropenia (a reduction in neutrophil numbers, particularly common as a result of chemotherapy) favours IA. Contact between neutrophils and hyphae triggers the secretion of reactive oxygen intermediates and enzymatic granule components. In contrast to the killing of conidia by macrophages, hyphal damage by PMN is rapid. As for AM, corticosteroids are also associated with a failure of the PMN phagocytic response in controlling *A. fumigatus* (Diamond & Clark, 1982 ; Levitz & Farrell, 1990 ; Rex *et al*., 1990).

As mentioned earlier, identification of the sites of infection and putative latency of this fungus in the lung compartment in the IC patient would markedly help our understanding of the pathobiology of the infection and could lead to efficient prophylactic interventions that have not been applicable to date for IA.

Analysis of fungal aggressiveness

Invasive aspergillosis results from the growth in the lung of a branched, septate vegetative mycelium that will invade the lung tissues. Virulence of *A. fumigatus* could then be due to fungal molecules that either promote mycelial growth specifically into the lung parenchyma or are responsible for a higher resistance of *A. fumigatus* to the debilitated antifungal mechanisms of the immunocompromised human host. These molecules should be absent from other thermophyllic airborne pathogens, a control always forgotten in the analysis of *A. fumigatus* virulence!

Tools and strategies to analyse fungal virulence

To date, the study of *A. fumigatus* virulence is based on the analysis of mutants constructed by gene disruption, and the detection of attenuated virulence of the mutant through an animal screen (Latgé, 2001). Two strategies have been used to investigate *A. fumigatus* virulence using mutants. In the first approach, putative virulence factors are selected in advance. This selection is based on the biochemical function of the protein, which should putatively help the invading mycelium to grow *in vivo*. The main drawback of this approach is that identification of the function of the protein is based on a fungus grown *in vitro*. The second approach has been the construction of libraries of mutants by random insertional mutagenesis. Early studies used the integration of a selection marker such as hygromycin (Brown *et al*, 2000) but the integration of a transposon is now most commonly used (Firon, 2002).

Animal models play a central role in the identification of a virulence factor. Although mice are the most commonly used test animals, there is no consensus about the best experimental model to use. Even though immunosuppressive treatments substantially increase the susceptibility of animals to infection, and intranasal inoculation mimics the natural route of infection and seems to be a more appropriate route than intravenous injection, an animal model has not yet been developed that effectively mimics chronic infection caused by the inhalation of a low number of conidia (Latgé, 1999). In the usual approach to the study of virulence, strains of *A. fumigatus* are inoculated into separate groups of animals and death is monitored over time. This method is only appropriate to identify mutant strains characterized by high differences in virulence. Models involving mixtures of strains are more useful in quantifying slight variations in strain aggressiveness not detected when isolates are inoculated individually (Sarfati *et al*., 2002).

Putative virulence factors of A. fumigatus

No true virulence factors have been identified to date in *A. fumigatus*. In particular, the catalase, proteases and toxin secreted by this fungus do not play a role in the pathogenesis of *A. fumigatus* in experimentally-induced infections. Genes identified to date as playing a role in disease development are shown in Table 2. PABA or PyrG auxotrophs are not able to germinate in the lung because the chemicals correcting auxotrophy are absent from the lung (Brown *et al*., 2000 ; d'Enfert *et al*., 1996). Strains with reduced growth rate, such as those obtained by disruption of the cell wall chitin synthase (Aufauvre-Brown *et al*., 1996) or glucanosyltransferase (Mouyna *et al*., submitted), are less pathogenic than wild type parental strains. The importance of growth rate over the total growth can be emphasized in revisiting a double protease mutant that we constructed a few years ago (Jaton-Ogay *et al*., 1994). When these mutants were plated on an agar medium with or without 1% BSA, the growth rate of the mutant and wild type was not modified, whereas the total mycelial dry weight was reduced by the double mutation (Fig 3). No difference in mortality was seen between the mutant and parental strain, confirming that the growth rate is a key factor for growth *in vivo*.

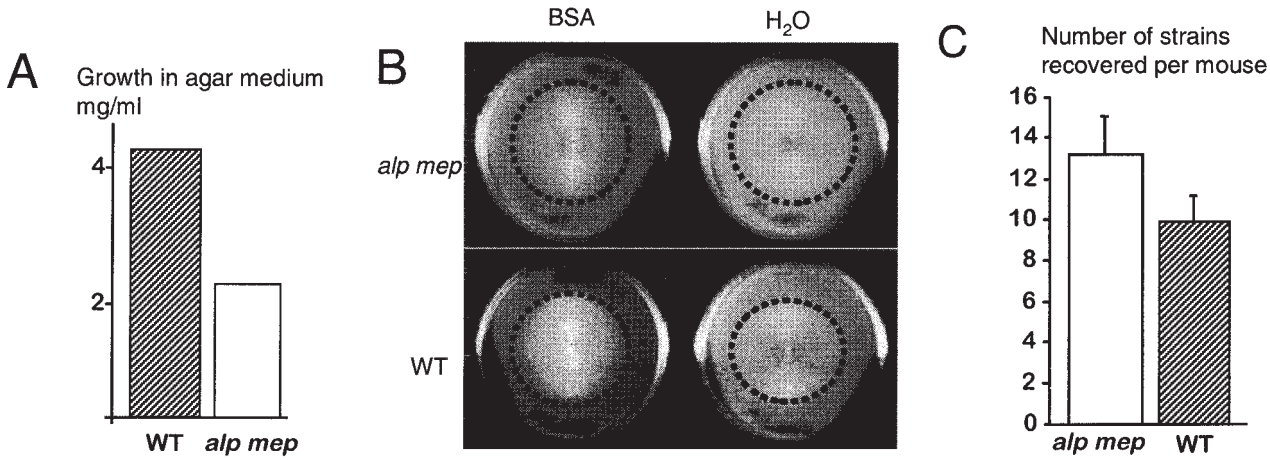

Fig 3 Growth and infectivity of a double alkaline serine protease/metalloprotease mutant (*alp mep*) and its parental strain (WT). A: note that in a medium supplemented with bovine serum albumin (BSA), total mycelial growth is higher in the wild type strain. B: hyphal extension is identical in mutant and parental strains in agar media with or without BSA (dotted line indicates colony diameter). C: note no difference of virulence is seen in a mixed model of infection between wt and *alp mep* strains.

The role of nutrient availability can also be correlated with low infectivity, for example in the case of the *areA* mutant, a global nitrogen regulatory gene in *Aspergillus* (Hensel *et al*., 1998).

Attenuated virulence has also been associated with the disruption of genes involved in the resistance of the fungus to the phagocytic immune response (Tsai *et al*, 1999; Jahn *et al*, 2000). Mutants with white conidia resulting from the inactivation of the *ALB1* gene, a polyketide synthase essential for the synthesis of dihydroxynaphtalene melanin in *A. fumigatus*, were more efficiently damaged by phagocytes than the wild-type. The melanin layer of the conidial cell wall efficiently scavenges reactive oxygen species and plays a major role in protection against the phagocytic reactions. The value of this observation can be questioned as it is extremely rare to isolate strains from the environment with white conidia. In addition, using an intravenous murine infection model, the virulence of white conidia was reduced by approximately 20-50% of that observed with wild-type, but non-pigmented conidia were still able to induce IA when inoculated intranasally into steroid-treated mice (Sarfati *et al*., 2002).

This observation led us to question whether all environmental strains of *A. fumigatus* have the same virulence potential and whether epidemiological studies could bring new insights into strain pathogenicity. No difference in growth was observed for hundreds of airborne strains analysed in our laboratory (unpublished observations). Strain variability was scrutinized more deeply using DNA fingerprinting methods such as Southern blot patterns of restricted DNA probed with retrotransposon-like elements such as *Afut1* and *Afut2*, or microsatellite length polymorphisms (Bart Delabesse *et al*. 2001). A survey of hundreds of isolates showed that as many as 85% of the environmental isolates investigated displayed unique genotypes, and that these strains were detected only once within a two year-period from the same environment (Chazalet *et al*. 1998). No particular strains were indeed isolated repeatedly from the same specific hospital. Patients suffering from IA or from aspergilloma are however usually infected by one or two strains only (Fig 4). In addition, when bronchial colonization occurs in the absence of invasive aspergillosis, e.g. in patients with cystic fibrosis, data gathered to date indicate that patients colonized by *A. fumigatus* usually harbour several strains that were found recurrently per patient (Neuvéglise *et al*., 1997).

The presence of one or two strains per patient with invasive and/or disseminated infections, despite the presence of thousands of genotypes from the environment potentially inhaled by the patient, could have two explanations (Fig 4). Firstly, infection is random and the strains that predominate are the first to grow at a time when the patient is the most susceptible. Secondly, clinical isolates are more virulent than environmental isolates. Typing of environmental and patients' isolates did not show any clustering among clinical and environmental strains (Debeaupuis *et al*. 1997) suggesting that every environmental strain should be considered a potential pathogen for any appropriate host at risk of infection. If every isolate of *A. fumigatus* is potentially pathogenic, then detection of one/two genotypes per patient would more likely reflect competition among isolates for infection or colonization at one point in time, rather than the

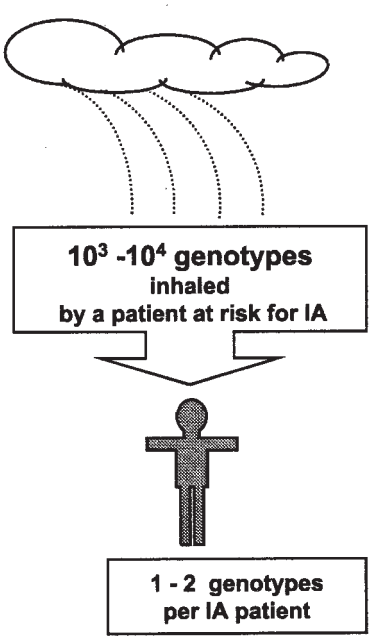

Fig 4 Epidemiological facts in invasive aspergillosis.

existence of isolates with different levels of virulence. Indeed, IA can be easily induced in animal models with any strain isolated from the environment or from a different host. However, recent data, using intravenous or intranasal assays with a mixture of different strains as an inoculum, have suggested that strains selected randomly from a laboratory collection have different pathogenicities since poorly infective strains could not be recovered (Sarfati *et al*. 2002). These preliminary data were unexpected and suggest that there are differences in virulence between environmental isolates. Such studies should be pursued with a higher number of strains, especially since two studies using other murine models of invasive aspergillosis have suggested clinical isolates to be more virulent than environmental isolates (Mondon *et al*., 1995; Aufauvre-Brown *et al*., 1998).

Conclusion

Single gene disruption studies have not been able to identify a true virulence factor for *A. fumigatus*, *viz*., a gene or a protein essential for growth *in vivo* whose deletion does not affect mycelial growth *in vitro*. It is then tempting to speculate that the virulence of *A. fumigatus* is directly correlated with its specific biological characteristics, namely the small size of conidia allowing their dispersal throughout the entire respiratory tract, rapid mycelial growth at temperatures around 37°C, and absence of specific nutritional requirements. However, the fact that *A. fumigatus* is responsible for in excess of 90% of human pulmonary mycosis would suggest that this fungal species displays some molecular features that favour its development in human tissues. Earlier studies have suggested that virulence of *A. fumigatus* may be polygenic; further analysis of *A. fumigatus* virulence would require the identification of global regulators or multiple genes expressed concomittantly in the same metabolic pathway. To date, the most efficient way to reach this objective is a comparative transcriptome and/or proteome analysis of the fungus grown *in vitro* and *in vivo*. Such an approach is now feasible since the sequence of the entire genome of *A. fumigatus*, although not annotated yet, is available at http://www.tigr.org/tdb/e2k1/afu1.

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 in the immuno-competent host, the molecular mechanisms debilitated by the immunotherapies remain to be elucidated. Similarly, studies of cross-talk 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 one wants to improve protective immunity against this fungal infection.

References

Aufauvre-Brown, A., Mellado, E., Gow, N. A. & Holden, D. W. (1996). *Aspergillus fumigatus* chsE: a gene related to CHS3 of *Saccharomyces cerevisiae* and important for hyphal growth and conidiophore development but not pathogenicity. *Fungal Genetics and Biology* **21**: 141-152.

Aufauvre-Brown, A., Brown, J. S., & Holden, D. W. (1998). Comparison of virulence between clinical and environmental isolates of *Aspergillus fumigatus*. *European Journal of Clinical Microbiology and Infectious Diseases* **17**: 778-780.

Bart-Delabesse, E., Sarfati, J., Debeaupuis, J. P., van Leeuwen, W., van Belkum, A., Bretagne, S. & Latgé, J. P. (2001). Comparison of restriction fragment length polymorphism, microsatellite length polymorphism, and random amplification of polymorphic DNA analyses for fingerprinting *Aspergillus fumigatus* isolates. *Journal of Clinical Microbiology* **39**: 2683-2686.

Beffa, T., Staib, F. & Lott Fischer, J. (1998). Mycology control and surveillance of biological waste and compost. *Medical Mycology* **36**: 137-145.

Brown, J. S., Aufauvre-Brown, A., Brown, J., Jennings, M. & Arst, H. (2000). Signature-tagged and directed mutagenesis identify PABA synthetase as essential for *Aspergillus fumigatus* pathogenicity. *Molecular Microbiology* **36**: 1371-1380.

Chazalet ,V., Debeaupuis, J. P., Sarfati, J., Lorthoraly, J., Ribaud, P., Shah, P., Cornet, M., Vu Thien, H., Gluckman, E., Brüker, G. & Latgé, J. P. (1998). Molecular typing of environmental and patient isolates of *Aspergillus fumigatus* from various hospital settings. *Journal of Clinical Microbiology* **36**: 1494-1500.

Cordonnier, C., Gilain, L., Ricolfi, F., Deforges, L., Girard-Pipau, F., Poron, F., Millepied, M. C. & Escudier, E. (1996). Acquired ciliary abnormalities of nasal mucosa in marrow recipients. *Bone Marrow Transplantion* **17**: 611-616.

Debeaupuis, J. P., Sarfati, J., Chazalet, V. & Latgé, J.P. (1997). Genetic diversity among clinical and environmental isolates of *Aspergillus fumigatus*. *Infection and Immunity* **65**: 3080-3085.

Denning, D. W. (1998). Invasive aspergillosis. *Clinical Infectious Disease* **26**: 781-805.

d'Enfert, C., Diaquin, M., Delit, A., Wuscher, N., Debeaupuis, J. P., Huerre, M. & Latgé, J. P. (1996). Attenuated virulence of uridine-uracil auxotrophs of *Aspergillus fumigatus*. *Infection and Immunity* **64**: 4401-4405.

Diamond, R. D. & Clark, R. A. (1982). Damage to *Aspergillus fumigatus* and *Rhizopus oryzae* hyphae by oxidative and non-oxidative microbicidal products of human neutrophils *in vitro*. *Infection and Immunity* **38**: 487-495.

Firon, A. (2002). Identification de gènes essentiels à la croissance du champignon pathogène de l'homme *Aspergillus fumigatus*. Thèse de Doctorat de l'Université Paris 7, soutenue le 17 Octobre 2002.

Fisher, G., Müller, T., Schwalbe, R., Ostrowski, R. & Dott, W. (2000). Exposure to airborne fungi, MVOC and mycotoxins in biowaste-handling facilities. *International Journal of Hygiene and Environmental Health* **203**: 37-104.

Heederik, D., Dowes, J., Wouter, I. & Doekes, G. (2000). Organic dusts: beyond endotoxins. *Inhalation Toxicology* **12**: 27-33.

Hensel, M., Arst, H. N., Aufauvre-Brown, A. & Holden, D. W. (1998). The role of the *Aspergillus fumigatus areA* gene in invasive pulmonary aspergillosis'. *Molecular Genetics* **258**: 553-557.

Jahn, B., Boukhallouk, F., Lotz, J., Langfelder, K., & Wanner, G. (2000). Interaction of human phagocytes with pigmentless *Aspergillus* conidia. *Infection and Immunity* **68**: 3736-3739.

Jaton-Ogay, K., Paris, S., Huerre, M., Quadroni, M., Falchetto, R., Togni, G., Latgé, J.P. & Monod, M. (1994). Cloning and disruption of the gene encoding an extracellular metalloprotease of *Aspergillus fumigatus*. *Molecular Microbiology* **14**: 917-928.

Lacey, J. (1996). Spore dispersal – its role in ecology and disease: the British contribution to fungal aerobiology. *Mycological Research* **100**: 641-660.

Latgé, J. P. (1999). *Aspergillus fumigatus* and aspergillosis. *Clinical Microbiology Review* **12**: 310-350.

Latgé, J. P. (2001). The pathobiology of *Aspergillus fumigatus*. *Trends in Microbiology* **9**: 382-389.

Levitz, S. M. & Farrel, T. P. (1990). Human neutrophil degranulation stimulated by *Aspergillus fumgiatus*. *Journal of Leukocyte Biology* **47**: 170-175.

Millner, P. D., Marsh, P. B., Snowden, R. B. & Parr, J. F. (1977). Occurence of *Aspergillus fumigatus* during compositing of sewage sludge. *Applied and Environmental Microbiology* **34**: 765-772.

Mondon, P., Thelu, J., Lebeau, B., Ambroise-Thomas, P. & Grillot, R. (1995). Virulence of *Aspergillus fumigatus* strains investigated by random amplified polymorphic DNA analysis. *Journal of Medical Microbiology* **42**: 299-303.

Neuvéglise, C., Sarfati, J., Debeaupuis, J. P., Vu Thien, H., Just, J., Tournier, G. & Latgé, J. P. (1997). Longitudinal study of *Aspergillus fumigatus* strains isolated from cystic fibrosis patients. *European Journal of Clinical Microbiology and Infectious Diseases* **16**: 747-750

Paris, S., Boisvieux-Ulrich, E., Crestani, B., Houcine, O., Taramelli, D., Lombardi, L. & Latgé, J. P. (1997). Internalization of *Aspergillus fumigatus* conidia by epithelial and endothelial cells. *Infection and Immunity* **65**: 1510-1514.

Philippe, B., Ibrahim-Granet, O., Prevost, M.C., Gougerot-Pocidalo, M. A., Sanchez Perez, M., Van dert Meeren, A. & Latgé, J. P. (2003). Killing of *Aspergillus fumigatus* by the alveolar macrophage is mediated by reactive oxidant intermediates. *Infection and Immunity* (in press).

Rex, J. H., Bennett, J. E., Gallin, J. I., Malech, H. L. & Melnick, D. A. (1990). Normal and deficient neutrophils can cooperate to damage *Aspergillus fumigatus* hyphae. *Journal of Infectious Diseases* **162**: 523-528.

Rylander, R. (1999). Indoor air-related effects and airborne (1-3) B-D glucan. *Environmental Health Perspectives* **107**: 501-503.

Sarfati, J., Diaquin, M., Debeaupuis, J. P., Schmidt, A., Lecaque, D., Beauvais, A. & Latgé, J. P. (2002). A new experimental murine aspergillosis model to identifiy strains of *Aspergillus fumigatus* with reduced virulende. *Japanese Journal of Medical Mycology* **43**: 203-213.

Sigsgaard, T., Bonefeld-Jorgensen E. C., Kjaegaard, S. K., Mamas, S. & Pedersen, O. F. (2000). Cytokine release from the nasal mucosa and whole blood after experimental exposures to organic dusts. *European Respiratory Journal* **16**: 140-145.

Travis, S. M., Conway, B. A., Zabner, J., Smith, J. J., Anderson, N. N., Singh, P. K., Greenberg, E. P. & Welsh, M. J. (1999). Activity of abundant antimicrobials of the human airway. *American Journal Respiratory Cell and Molecular Biology* **20**: 872-879.

Tsai, H. F., Chang, Y. C., Washburn, R. G., Wheeler, M. H. & Kwon-Chung, K. J. (1999). The developmentally regulated gene cluster involved in conidial pigment biosynthesis in *Aspergillus fumigatus*. *Journal of Immunology* **181**: 6469-6477.

Wasylnka, J. A. & Moore, M. M. (2002). Uptake of *Aspergillus fumigatus* conidia by phagocytic and non phagocytic cells *in vitro*: quantitation using strains expressing green fluorescent protein. *Infection and Immunity* **70**: 3156-3163.