REVIEWS IN MEDICAL MICROBIOLOGY

Aspergilli – significance as pathogens

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Summary. *Aspergillus* species are widespread in the environment and have the capacity to cause life-threatening invasive disease, notably in the immunocompromised patient. In this review, an initial consideration of environmental sources of aspergilli will be followed by examination of the significance of *Aspergillus* isolated from human cultures. A review of the various disease patterns seen in the immunocompetent and immunocompromised leads on to discussion of what is known about pathogenesis and determinants of virulence. Finally, to give some idea of the scale of the problem, we consider the occurrence of three types of disease due to aspergilli, namely, allergic bronchopulmonary aspergillosis, pulmonary aspergilloma, and invasive aspergillosis.

Introduction

Aspergillus spp. are saprophytes which are ubiquitous in nature and found worldwide. Over 250 species of *Aspergillus* have been recognised, but only a few regularly cause human or animal disease. Invasive aspergillosis is a well-recognised complication of therapeutic immunosuppression, and has increased dramatically in incidence over recent years. It is, however, only one of several diverse disease patterns caused by aspergilli. This diversity of clinical manifestations prompts examination of the role of aspergilli as human pathogens, in both immunosuppressed and immunocompetent individuals.

Ecology: *Aspergillus* in the environment

Aspergilli are common saprophytes in the human environment, especially in the autumn and winter in temperate climates in the Northern hemisphere, a fact reflected in more frequent episodes of human illness during winter months.[1] Together with *Penicillium* spp., the aspergilli make up 1–6% of the total flora of outside air; *A. fumigatus* makes up 4–41% of the total fungi cultured.[2] The usual concentration of spores in the air is 2–15 per cubic metre.

Air contamination of private houses by fungal spores is an important cause of human exposure. Such contamination also occurs in the hospital environment, causing outbreaks of nosocomial aspergillosis. In the home, indoor dusts are often rich in organic material and act as reservoirs of microfungi. This is particularly important in mattresses, where warm humid conditions may permit fungi to grow. Damp cellars and other dusty spaces are similarly heavily contaminated. Other sources of aspergilli, including species pathogenic for man, include the soil of potted plants[3,4] and condiments, especially ground black pepper.

A. fumigatus contamination of air conditioning systems causes emission of spores in the environment of hospitalised immunocompromised patients, leading to nosocomial outbreaks. These are much more likely to occur in the context of recent building work.

Isolation of aspergilli from human cultures

The most common species of *Aspergillus* causing allergic and invasive disease is *A. fumigatus*, but *A. flavus* has been recognised as an important pathogen in recent years. Other less common pathogenic aspergilli include *A. glaucus*, *A. niger*, *A. terreus*, and rarely, *A. nidulans*. *A. niger* is the most common cause of otomycosis. Only *A. fumigatus* has been typed for epidemiological purposes and DNA

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fingerprinting suggests that a limited number of clones are present worldwide.[5]

Aspergillus spp. may be present as commensals, most commonly in the respiratory tract, in normal individuals, though it is difficult to define the precise frequency with which aspergilli are recovered from a truly 'normal' population. Certainly aspergilli can be isolated from the oropharynx and gastrointestinal tract of normal hosts: Finnish workers recovered *Aspergillus* spp. from the tonsils of 11 of 147 patients undergoing tonsillectomy and from 5 of 134 patients undergoing adenoidectomy.[6] Another group recovered the genus from 17 of 23 throat culture specimens, 4 of 26 jejunal cultures, 15 of 20 ileal cultures, and 12 of 17 colonic cultures from healthy individuals.[7] *A. niger* was the most common isolate; no specimens yielded *A. fumigatus* or *A. flavus*.[7]

The significance of aspergilli cultured from sputum is more difficult to assess. In various studies, the rate of recovery of *Aspergillus* from healthy people has ranged from 0.5 to 16%.[8] Chronic lung disease increases the frequency of colonisation but again there is considerable variation in the rates reported. In one British study, *Aspergillus* spp. were cultured from sputum specimens of 7% of 2080 hospital inpatients with various chest problems: some of these individuals demonstrated hypersensitivity to aspergilli but none had invasive disease.[9] This contrasts with a colonisation rate of 57% in one group of patients with cystic fibrosis.[10] Factors likely to account for the wide variation in rates of colonisation with aspergilli include the patient population studied; the frequency with which sputum samples are obtained; the microbiological bias in studies specifically designed to isolate *Aspergillus* spp., and regional variation in the concentration of airborne spores.

In general, the majority of patients in an unselected hospital population with *Aspergillus* in their sputum do not have invasive aspergillosis, as was neatly demonstrated in a prospective study of hospital inpatients by Yu et al.[11] All 108 patients with sputum cultures positive for aspergilli were followed over a 3-year period. Invasive aspergillosis was not found in any of the immunocompetent patients.

However, this study also demonstrated that in patients at risk of invasive aspergillosis, positive sputum cultures are highly significant: all 17 of the sputum-positive patients with leukaemia and/or neutropenia who underwent lung biopsy were shown to have invasive aspergillosis. Similar results have been obtained from studies of other groups at risk of invasive disease, including heart transplant recipients[12] and renal transplant recipients,[13] in the pre-cyclosporin era. These studies need repeating now that corticosteroid use has declined in most of these patients. Allogeneic bone marrow transplant patients, no longer neutropenic, who have a positive culture for *Aspergillus* spp., should be presumed to have invasive aspergillosis until proven otherwise as the mortality of the disease currently exceeds 90%. In lung transplant patients, tracheobronchial aspergillosis has been described with no symptoms and normal chest X-ray,[14] thus a positive *Aspergillus* culture should prompt immediate bronchoscopy. In liver transplant patients, respiratory cultures positive for aspergilli had a sensitivity of 81% and specificity of 99% for invasive aspergillosis.[15] Invasive aspergillosis is an uncommon complication in patients with AIDS. Recovery of *Aspergillus* from respiratory sites in such patients usually reflects colonisation and is rarely associated with invasive disease, unless the patient is also neutropenic.[16]

Only a minority of patents, between 8 and 34%, who are later shown to have invasive aspergillosis will have aspergilli in their sputum ante mortem;[17,18] thus a negative sputum has low predictive value. The important clinical message is that the isolation of *Aspergillus* spp. (particularly *A. fumigatus* and *A. flavus*) from the respiratory secretions of an at-risk patient, on even a single occasion, is highly predictive of invasive disease and should never be dismissed.[19]

Pathogenesis and determinants of virulence

Pathogenesis

The disease patterns caused by aspergilli may be grouped into three categories (Table). In the normal host, colonisation may lead to hypersensitivity phenomena, such as allergic bronchopulmonary aspergillosis or allergic sinusitis. The immunocompetent may also develop superficial skin infections, and localised aspergillus infection in paranasal sinuses or

Table Classification of Aspergillus infection

Disease in the normal host
Hypersensitivity syndromes
Extrinsic asthma
Extrinsic allergic alveolitis
ABPA
Superficial infection
Cutaneous infection
Otomycosis
Sinusitis
Invasive infection
Single organ or disseminated
Infection associated with tissue damage or foreign body
Keratitis and/or endophthalmitis
Burn wound infection
Osteomyelitis
Prosthetic valve endocarditis
Aspergilloma
Empyema and pleural aspergillosis
Hickman or intravenous line associated invasive aspergillosis
Infection in the immunocompromised host
Primary cutaneous aspergillosis
Sino-orbital infection
Pulmonary aspergillosis:
invasive/chronic necrotising aspergillosis
Tracheobronchial aspergillosis:
obstructing bronchial aspergillosis
invasive aspergillus tracheobronchitis
Disseminated aspergillosis

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a pre-existing lung cavity (aspergilloma). Invasive aspergillosis is almost always seen in the context of the immunocompromised host; the lung is the commonest focus of infection, but aspergillosis may arise in the nose or paranasal sinuses, the bronchial tree or trachea or the skin. Thereafter dissemination to almost any body site may occur, particularly the brain.

Aspergillus spp. are primarily respiratory pathogens, hence most infections involve the paranasal sinuses and lungs. As we have seen, conidia are frequently present in the air we inhale, but only a few individuals develop invasive aspergillosis. The aspergillus spores are 2–3 μm in diameter, an ideal size to penetrate the pulmonary defences and reach the alveolar air space. In the normal host, spores encounter pulmonary macrophages, which are able to prevent germination and kill conidia by non-oxidative mechanisms.[20] In patients with impaired pulmonary macrophage function, most commonly due to corticosteroid therapy or immunosuppression post-organ transplant, conidia may germinate and cause invasive pulmonary aspergillosis (IPA). Various patterns of IPA are described, including pseudomembranous tracheobronchial aspergillosis; haemorrhagic infarction; nodules which may or may not cavitate, and a miliary pattern. Histological examination of the lung in IPA often reveals microcolonies of aspergillus surrounded by very extensive areas of necrosis, suggesting that extracellular products of the fungus – toxins or enzymes – are important in the pathological process.[21] Probably a combination of organism-related and host-related factors account for the diversity of clinical patterns seen in IPA.

After the conidia have germinated, it is the neutrophil that is most active in killing hyphae, with some contribution from monocytes. Hence neutropenic patients are at considerable risk of developing aspergillosis; 70% of patients after 30 neutropenic days according to one study by Gerson et al.[22] Without reversal of neutropenia, virtually all cases of invasive aspergillosis have been fatal.

Environmental exposure to *Aspergillus* spores is the major cause of invasive pulmonary aspergillosis, but there is also evidence that reactivation of endogenous organisms may produce significant infection in the immunocompromised. In patients rendered neutropenic by chemotherapy, aspergillus infection may resolve, only to reactivate when the patient is again neutropenic, and cause clinically apparent infection.[23]

Determinants of virulence

Various factors have been postulated as determinants of virulence of *Aspergillus* spp. Most experimental work has focused on *A. fumigatus* and important characteristics are thought to include its small spore size, rapid growth rate, and proteolytic activity, all of which confer increased virulence in murine models. The ability to produce proteolytic enzymes including elastase (which correlated with high mortality in a murine respiratory model)[24] and toxins is also likely to contribute to the capacity of *A. fumigatus* to cause disease in man.

Many questions regarding pathogenesis remain unanswered, but three observations make it likely that some or all of these factors are important in contributing to disease. First, the vast majority of *Aspergillus* spp. are not pathogenic, suggesting that the pathogenic few have special properties. Secondly, many different patterns of disease (X-ray manifestations, pace of disease, pattern of dissemination) are seen in the same host group, implying that organism-related factors are important. Thirdly, there are major dfferences between isolates by antigen or DNA typing, thus suggesting variations in virulence among isolates.[25]

The scale of the problem: how common is disease due to *Aspergillus*?

To try to gain an overview of the importance of *Aspergillus* spp. as human pathogens, it is instructive to compare the incidence of three forms of aspergillus infection, namely allergic bronchopulmonary aspergillosis, aspergilloma, and invasive aspergillosis.

Allergic bronchopulmonary aspergillosis (ABPA)

This is a condition that was first described in the UK. It is an inflammatory disease of the airways characterised by asthma, eosinophilia, infiltrates on the chest X-ray, raised serum IgE levels, circulating precipitating antibodies, and immediate skin test reactivity to *Aspergillus*. It is almost always caused by *A. fumigatus*, which can be isolated from sputum cultures. It is much more common than initially suspected, and may occur in up to 20% of asthmatics,[26,27] depending on the criteria used for diagnosis.

The pathogenesis of ABPA is not entirely clear. The initial step is trapping of aspergillus spores in the thick endobronchial secretions of the asthmatic. The fungus grows releasing toxins and antigens. The recently described production, by *A. fumigatus*, of a low-molecular-weight diffusate capable of interfering with lung phagocyte activity, is probably important at this stage.[28] Sensitisation occurs, causing a variety of immune reactions resulting in granuloma formation with mononuclear infiltration.[29] Ultimately recurrent attacks of inflammation lead to fibrosis, proximal bronchiectasis, and respiratory failure.[30] Early steroid treatment alleviates symptoms and may prevent progressive lung damage, so it is important that ABPA is recognised.

In this setting, *A. fumigatus* is an indolent pathogen, causing disease because of the aggressive immune response elicited in susceptible individuals.

Pulmonary aspergillomata

Patients with a pre-existing cavity in the lung are vulnerable to a localised aspergillus infection, or aspergilloma. A fungus ball is formed when spores are deposited in the cavity and germinate on the wall where mycelia and debris attach to form an amorphous mass. Disease processes which damage the lung and predispose to aspergilloma formation include tuberculosis (by far the commonest cause of an apical cavity), sarcoidosis, ankylosing spondylitis, histoplasmosis, pyogenic lung abcess, bronchiectasis, and pulmonary infarction. Aspergillomata may arise as a late complication of controlled IPA.

Some idea of the prevalence of aspergilloma can be gained from a reported review of 60000 chest radiographs: aspergillomata were identified in 0.01%.[31] During an 11-year period, 15 patients with aspergilloma were admitted to a Veteran's Administration hospital, representing 0.02% of admissions.[32]

Again, in this setting, aspergilli cause an indolent infection, and indeed, spontaneous resolution of the fungus ball is recognised to occur in 10% of cases within 3 years.[33] However, the consequence of this type of aspergillus infection can be dramatic. One report focused on the long-term outcome of 23 patients with aspergilloma, 5 of whom died from complications of their infection.[34]

Invasive aspergillosis

Invasive aspergillosis is endemic in some institutions and sporadic in others. Thus individual estimates of incidence may fail to illustrate the problem. In many centres treating leukaemia with or without bone marrow transplantation, fungal infection and particularly invasive aspergillosis is the leading cause of death. In a European survey, 9 of 33 (27%) centres had an estimated 13 or more cases of suspected invasive aspergillosis annually, more cases occurring in larger hospitals.[35] Among 2180 liver transplant recipients in one US centre, 2% had cultures positive for aspergilli. Three-quarters of these had invasive infection and mortality was over 90% despite aggressive antifungal therapy.[15] At Stanford University Hospital some 13% of heart transplant recipients developed invasive aspergillosis.[36] At the Christie Hospital in Manchester, of 42 autopsies in leukaemic patients from 1988 to 1991, 13 (33%) had invasive aspergillosis. Sometimes invasive aspergillosis is not the direct cause of death; patients died 'with aspergillosis' rather than 'of aspergillosis', but the problem is generally a common one in this group of patients.

Invasive aspergillosis has a high mortality. Cerebral aspergillosis and pulmonary or disseminated aspergillosis in bone marrow transplant patients carry mortality rates exceeding 90%.[36] Very few patients with AIDS and invasive aspergillosis have responded to therapy.[37,38] The overall success rate of therapy is probably 30–35% but this figure rises to 55% if patients are able to receive at least 2 weeks of antifungal therapy.[36] Recurrence during subsequent courses of chemotherapy or bone marrow transplantation is common and may delay or indefinitely postpone these procedures, preventing cure of the underlying leukaemia or lymphoma.[23]

Conclusion

Given the enormous increase in numbers of immunocompromised patients worldwide and the propensity of *Aspergillus* spp. to cause life-threatening disease, the significance of this organism should not be underestimated. Clinicians should have a high index of suspicion when treating immunocompromised patients and isolation of *Aspergillus* spp. from such patients should prompt a rapid diagnostic assessment and usually, the immediate institution of therapy.

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