REVIEW ARTICLE

MEDICAL PROGRESS

Aspergillosis

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Filamentous fungi (molds) are ancient lineages that have existed for approximately 1 billion years[1] and thrive in soil and decomposing vegetation independent of an animal host. Thus, the evolution from primitive immune systems that rely principally on antimicrobial peptides, such as those in insects,[2] to the complex immune system in mammals occurred with continued exposure to fungi. The immune system, therefore, must not only recognize inhaled molds and control their growth but also restrain injurious inflammation and allergy.

We regularly inhale the spores of aspergillus species, yet fungal disease is uncommon. Aspergillus-related diseases are associated with a spectrum of disorders of immunity. Invasive aspergillosis, the focus of this review, is typically a disease of highly immunocompromised persons and is a leading cause of infection-related death in patients with acute leukemia and recipients of allogeneic hematopoietic stem-cell transplants. At the other end of the immunologic spectrum, allergic forms of aspergillosis, such as allergic bronchopulmonary aspergillosis, result from a poorly controlled inflammatory response to hyphae colonizing the sinopulmonary tract. Important advances have been made in fungal diagnostics and in the antifungal armamentarium. In addition, new insights have been gained regarding host defense against aspergillus species and the immunopathogenesis of aspergillus-related diseases that may pave the way to new prevention and therapeutic strategies.

Host Defense against Aspergillus

Innate Immunity

Respiratory epithelial cells act as an anatomic barrier to invasion by inhaled aspergillus species, promote mucociliary clearance, and ingest inhaled conidia (spores). The ability of aspergillus species to survive within epithelial cells may enable evasion of host defense by phagocytes.[3] Alveolar macrophages constitute the first line of phagocytic host defense against inhaled conidia.[4] Peripheral-blood monocytes and neutrophils are subsequently recruited to sites of infection. After fungal germination (transformation from conidia to hyphae), neutrophils are the dominant host defense against hyphae, the tissue-invasive form of molds.[4] Natural killer cells are recruited to the lungs by chemokines early in experimental aspergillosis and play an important host-defense function.[5]

The NADPH oxidase in phagocytes is essential in host defense against aspergillosis, as demonstrated in patients with chronic granulomatous disease, an inherited disorder of NADPH oxidase. Chronic granulomatous disease is associated with recurrent bacterial and fungal diseases, and invasive aspergillosis is a major cause of death in patients with this disease.[6] The activation of NADPH oxidase results in the conversion of oxygen to superoxide anion and the generation of downstream reactive oxidant metabolites with antimicrobial activity. In neutrophils, the activation of NADPH oxidase is coupled with activation of antimicrobial proteases sequestered

in primary granules.[7] The neutrophil NADPH oxidase is activated by constituents of the fungal-cell wall and is required to induce hyphal damage. In contrast, neutrophil-mediated inhibition of conidial growth is independent of NADPH oxidase but requires lactoferrin-mediated iron depletion, illustrating the stage-specific antifungal activity of neutrophil-regulated host defense.[8] In addition to its host-defense function, NADPH oxidase has a counterbalancing role to restrain inflammation induced by fungal-cell-wall constituents,[9,10] a function that is probably mediated in part by the activation of tryptophan catabolism.[10]

Pathogen-recognition receptors in the host identify specific microbial motifs, such as cell-wall products (e.g., endotoxin produced by gram-negative bacteria and beta-glucans produced by fungi) and microbial DNA and RNA, and trigger innate immune responses. Several classes of cell-associated and soluble pathogen-recognition receptors that identify fungal motifs include toll-like receptors (TLRs), dectin-1, surfactant proteins A and D,[11] mannose-binding lectin,[12] and pentraxin-3[13] (Fig. 1). TLRs are a conserved family of pathogen-recognition receptors that are homologous to interleukin-1 receptor 1 and share a similar signaling cascade, leading to the activation of transcriptional factor nuclear factor κB (NF-κB) and mitogen-activated protein kinases. Activation of TLRs, in general, induces the expression of proinflammatory cytokines. Studies in TLR-deficient mice have shown that specific TLRs recognize different fungal motifs and regulate the inflammatory response in aspergillosis.[14-16] In addition, the stimulation of specific TLRs can modulate human neutrophil antifungal activity.[14]

Dectin-1 is a receptor and immunomodulator of beta-glucans, which are ubiquitous cell-wall constituents of fungi and plants. Dectin-1 is a natural-killer-cell–receptor-like C-type lectin that is expressed at high levels within the pulmonary and gastrointestinal tract, where exposure to pathogens regularly occurs.[17] Dectin-1 and TLR2 recognize distinct beta-glucan motifs and stimulate proinflammatory cytokine production.[18] Ligation of dectin-1 may stimulate NADPH oxidase activation.[18]

TLRs and dectin-1 permit host cells to distinguish between resting conidia, germinating conidia, and hyphae of *Aspergillus fumigatus*. Fungal beta-glucans trigger inflammatory responses in macrophages through their time-dependent exposure on the surface of germinating aspergillus conidia.[19-21] Coordinated ligation of specific pathogen-recognition receptors through the identification of stage-dependent fungal motifs probably calibrates the immune response to contain fungal growth while avoiding excessive inflammation after inhalation of ubiquitous aspergillus spores.

Myeloid differentiation factor 88 (MyD88) is a downstream adapter for most TLRs. The work of von Bernuth et al.[22] showed that children with autosomal recessive MyD88 deficiency have life-threatening bacterial infections but not other infectious complications. These findings suggest that a redundancy in antifungal host-defense pathways exists and that TLR-independent pathways may be sufficient for protection. However, TLR signaling is probably important in defense against aspergillosis for patients in immunocompromised states, such as occurs after transplantation.[23]

CELLULAR IMMUNITY

The activation of pathogen-recognition receptors generally induces maturation of antigen-presenting cells that prime T-cell immunity. Interferon-γ is the signature cytokine of type 1 helper T cells (Th1) and stimulates cellular immunity. CD4+ T-cell differentiation during experimental aspergillosis occurs in stages, with TLR-independent signals promoting Th1 differentiation in the lung and priming of TLR-dependent Th1 occurring in lymph nodes.[24] In murine aspergillosis, augmentation of Th1 responses by administration or depletion of specific cytokines enhances antifungal host defense.[25] In patients with invasive aspergillosis, increased Th1 versus type 2 helper T-cell (Th2) cytokine responses were associated with improved outcomes.[26,27]

Th2 cells produce interleukin-4, interleukin-5, and interleukin-13 and are implicated in allergy. Allergic bronchopulmonary aspergillosis develops from sensitization to bronchial airway *A. fumigatus* antigens that prime Th2 responses.[28] Suppression of Th2 responses attenuates experimental allergic bronchopulmonary aspergillosis.[28] Interleukin-17–producing CD4+ T cells are a distinct subgroup of T helper cells that are implicated in autoimmune diseases.[29] Interleukin-17 stimulates the production of specific myelopoietic growth factors and cytokines and chemokines that promote neutrophil recruitment, but paradoxically, interleukin-17 can impair host defense in experimental aspergillosis.[10,30] Regulatory T cells have a coun-

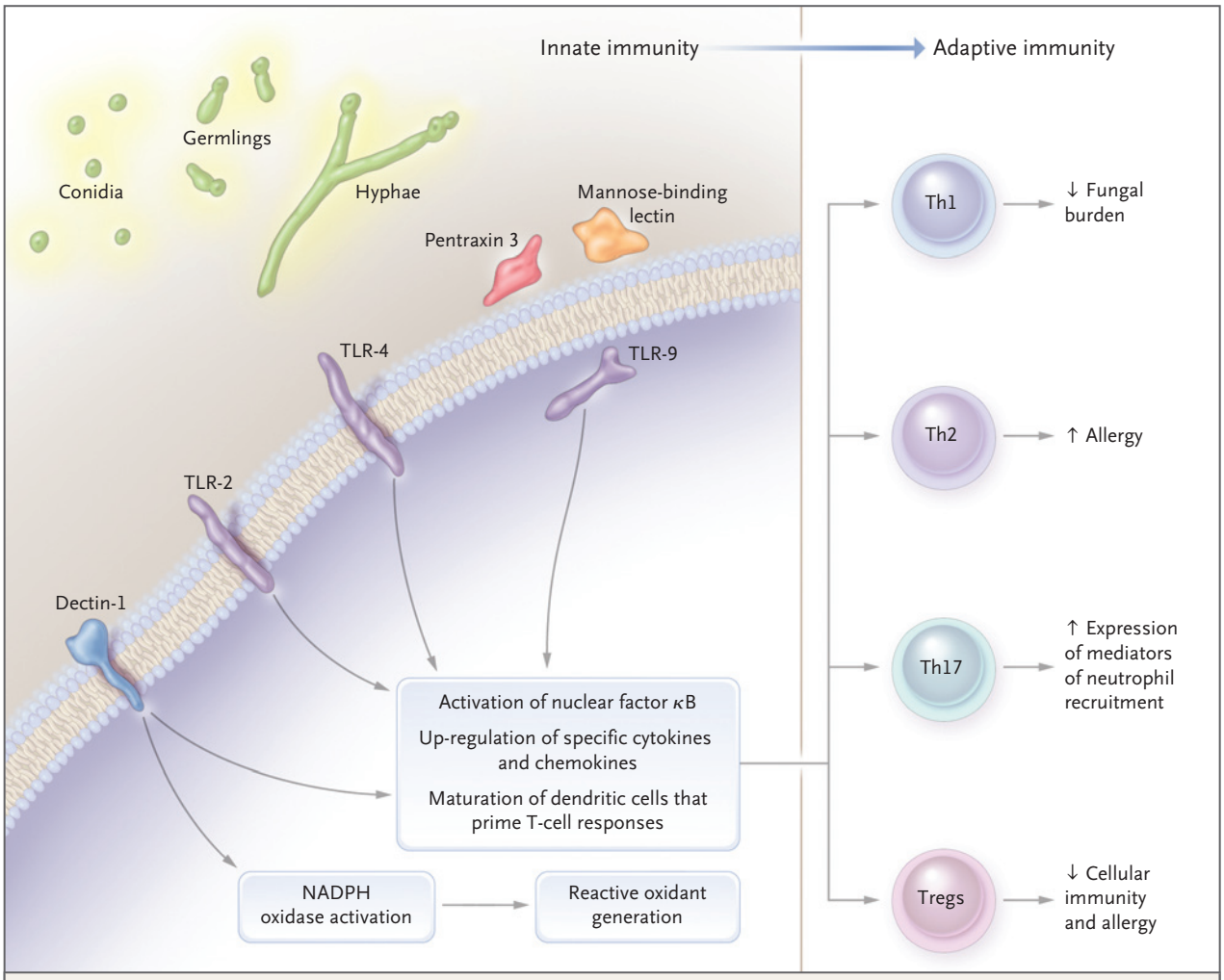


Figure 1. Model of Immune Response to Inhaled Aspergillus Species.

Alveolar macrophages and epithelial cells are the first host cells in the lung to engage inhaled aspergillus conidia. Pathogen-recognition receptors on host cells, such as dectin-1 and toll-like receptors (TLRs), and soluble pathogen-recognition receptors, such as pentraxin 3 and mannose-binding lectin, recognize specific fungal motifs. For example, fungal-cell-wall–associated beta-glucans ligate TLR-2 and dectin-1, and aspergillus DNA contains unmethylated CpG sequences that ligate TLR-9. Ligation of pathogen-recognition receptors generally leads to induction of chemokines and cytokines that activate and recruit neutrophils and other inflammatory cells. NADPH oxidase can be activated by dectin-1 and potentially primed by other pathogen-recognition receptors such as TLR4. NADPH oxidase activation leads to generation of reactive oxidant intermediates and in neutrophils is coupled to the activation of antimicrobial granular proteases. Dendritic cells also sense aspergillus motifs through pathogen-recognition receptors and stimulate antigen-dependent responses in helper T cells (Th) and regulatory T cells (Tregs). The cross-talk between dendritic cells and T cells in regulating T-cell development is an area of intensive research interest that is broadly relevant to host defense, allergy, and vaccine development. Most of the data in this model are derived from in vitro studies and animal models of aspergillosis.

terregulatory function by inducing tolerance and protecting against allergy in experimental aspergillosis.[31] The balance between the promotion and the suppression of specific classes of cytokines and chemokines and between the expansion and the contraction of specific T-cell populations modulates the extent and nature of the immune response to aspergillus species. This response, in turn, mediates control of fungal burden and allergy versus tolerance (Fig. 1).

SPECTRUM OF HUMAN DISEASE IN ASPERGILLOSIS

Aspergillosis encompasses a spectrum of diseases related to host factors (Table 1).[32-37] Acute invasive

Table 1. Predisposing Host Factors and Clinical and Histologic Features Associated with Invasive Pulmonary Aspergillosis.*

Population of Patients	Predisposing Host Factors	Clinical and Histologic Features
Acute leukemia, myelodysplastic syndrome, aplastic anemia, and other causes of marrow failure	Neutropenia from chemotherapy or underlying hematologic disease	Hyphal angioinvasion with vascular thrombosis and tissue infarction; scant inflammatory response; possible evolution to cavitation
Allogeneic hematopoietic stem-cell transplantation after neutrophil recovery	Immunosuppression for GVHD (e.g., the use of corticosteroids, T-cell depletion, inhibition of tumor necrosis factor α)	Inflammatory fungal pneumonia and angioinvasion with coagulative necrosis, which are classically associated with neutropenia[32-34]
Solid-organ transplantation	Immunosuppression to prevent allograft rejection	Acute inflammatory pneumonia, chronic necrotizing aspergillosis; tracheobronchitis affecting the anastomotic site and causing dehiscence in lung-transplant recipients
Advanced AIDS	CD4+ T-cell count usually <100 per cubic millimeter; other immunocompromising conditions (e.g., neutropenia)	Acute to slowly progressive necrotizing pneumonia; variable histologic findings: neutrophilic infiltrates, vascular invasion, walled-off abscesses, and cavitation[35]
Chronic granulomatous disease	Defective NADPH oxidase	Acute to slowly progressive pneumonia; pyogranulomatous inflammation without hyphal vascular invasion or coagulative necrosis; "mulch pneumonitis," an acute hypersensitivity response to a large, aerosolized exposure[36]
Preexisting structural lung disease (e.g., emphysema, previous cavitary tuberculosis)	Coexisting conditions (e.g., diabetes and malnutrition) and the use of inhaled and systemic corticosteroids	Chronic necrotizing pulmonary aspergillosis; slowly progressive invasive fungal pneumonia with inflammatory necrosis[37]

* AIDS denotes acquired immunodeficiency syndrome, and GVHD graft-versus-host disease.

aspergillosis is a rapidly progressive, frequently fatal disease that occurs in highly immunocompromised persons. In contrast, chronic forms of pulmonary aspergillosis (e.g., chronic necrotizing pulmonary aspergillosis or fibrocavitary aspergillosis) typically occur in patients without severe immune impairment, progress over months to years, and require prolonged antifungal therapy.[38,39] Aspergilloma is a fungal mass that develops in a preexisting lung cavity. Medical therapy is of uncertain value for aspergillomas; in cases of persistent hemoptysis, surgical resection of the infected lung cavity, if feasible, is the definitive therapy.[40]

In immunocompetent persons, aspergillus species can induce allergic responses, manifesting as sinusitis and asthma. Allergic bronchopulmonary aspergillosis is a severe Th2-mediated allergic disease that occurs in 1 to 2% of patients with asthma and in 1 to 15% of patients with cystic fibrosis.[28] Saprophytic hyphal elements that colonize bronchial airways cause recurrent inflammation and airway plugging, transient pulmonary infiltrates, and central bronchiectasis as a long-term consequence.

INVASIVE ASPERGILLOSIS

EPIDEMIOLOGY

Patients who are at risk for invasive aspergillosis include those with prolonged neutropenia, recipients of hematopoietic stem-cell transplants or solid-organ transplants, and patients with advanced acquired immunodeficiency syndrome or chronic granulomatous disease.[41] Host factors govern both the risk and clinicopathologic features of invasive aspergillosis (Table 1).[32-34] The number of aspergillosis-related deaths increased by a factor of four in the 1980s and 1990s in the United States on the basis of autopsy reports,[42] a reflection of an increased number of immunocompromised patients.

In patients with neutropenia, the degree and duration of neutropenia predict the risk of invasive aspergillosis. Intensive cytotoxic chemotherapy (e.g., induction regimens for acute leukemia) causes prolonged neutropenia. The incidence of invasive aspergillosis in patients with acute leukemia was approximately 5% in a multicenter European registry.[43] Patients with refractory leukemia who are treated with multiple cycles of cytotoxic chemo-

therapy (and who may have aplasia from the underlying cancer) are at particularly high risk for aspergillosis.

In recipients of allogeneic hematopoietic stem-cell transplants, three periods of risk for invasive aspergillosis and other mold diseases occur: first, neutropenia after the conditioning regimen; second, acute graft-versus-host disease (GVHD); and third, chronic GVHD (occurring at least 100 days after transplantation).[44] Invasive aspergillosis occurs more often during GVHD than during neutropenia in recipients of allogeneic hematopoietic stem-cell transplants, with the severity of GVHD and the intensity of immunosuppressive therapy being the principal risk factors.[45-47]

The degree of disparity between the donor and the recipient in human leukocyte antigen (HLA) is the major predictor of the incidence and the severity of GVHD. In a prospective U.S. surveillance study, the aggregate cumulative incidence of invasive aspergillosis at 12 months after allogeneic hematopoietic stem-cell transplantation was 2.3% with an HLA-matched related donor (providing the highest level of HLA donor–recipient concordance), 3.2% with an HLA-mismatched related donor, and 3.9% with an unrelated donor.[48] In contrast, the incidence of invasive aspergillosis was 0.5% after autologous hematopoietic stem-cell transplantation. Similar results were observed in a multicenter Italian registry, with aspergillosis being more common and more likely to be fatal in recipients of allogeneic hematopoietic stem cells than in recipients of autologous hematopoietic stem cells.[49] T-cell depletion of allografts to prevent GVHD impairs reconstitution of cellular immunity and increases the risk of invasive aspergillosis and cytomegalovirus infection.[50]

Among recipients of solid-organ transplants, the incidence of invasive aspergillosis is highest after lung transplantation.[48] Aspergillus species that colonize the airways in end-stage lung disease may be a source of fungal infection after single-lung transplantation.[51] In recipients of solid-organ transplants, late-onset invasive aspergillosis (occurring 3 months after transplantation) is becoming increasingly recognized and is associated with the intensity of immunosuppression to treat allograft rejection[52] and retransplantation.[53] In patients who have not undergone transplantation, intensive immunosuppressive regimens, such as those used to treat serious autoimmune disorders (e.g., vasculitis), can rarely be complicated by invasive aspergillosis.[54,55]

There is also a growing appreciation of invasive pulmonary aspergillosis in patients without classic risk factors, such as critically ill patients without documented immunodeficiency.[56] Research on these patients may identify unrecognized host-defense pathways against aspergillosis.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Invasive aspergillosis principally involves the sinopulmonary tract, reflecting that inhalation is the most common route of entry of aspergillus spores; other entry sites such as the gastrointestinal tract and skin occur on rare occasions. Asymmetric facial swelling, epistaxis, proptosis and cranial-nerve abnormalities (reflecting orbital disease or cavernous sinus involvement), ischemia of the palate, and bone erosion are signs suggestive of invasive fungal sinusitis. Fever, cough, and dyspnea are frequent although nonspecific findings of pulmonary aspergillosis; the lung is the most common site of invasive aspergillosis. Vascular invasion may manifest as pleuritic chest pain from pulmonary infarction or as hemoptysis. Poorly controlled infection may lead to extension to mediastinal and chest-wall structures and hematogenous dissemination that can involve virtually any organ. Involvement of the central nervous system is a devastating consequence of disseminated aspergillosis and may be manifested by seizures or focal neurologic signs from mass effect or stroke.

Diagnosis of invasive aspergillosis remains difficult in that clinical manifestations are not specific; radiologic findings can be suggestive but none are pathognomonic, and cultures of respiratory samples lack sensitivity. Histologic demonstration of invasive hyphae or a positive culture from a normally sterile environment (e.g., pleural fluid) represents proven invasive fungal disease. Newer antigen-based assays facilitate the diagnosis of probable invasive aspergillosis and can obviate the need for an invasive procedure. The diagnosis of probable invasive aspergillosis requires a combination of host factors (e.g., prolonged neutropenia and organ transplantation), compatible radiologic findings, and mycologic criteria.[57] These diagnostic criteria, though designed for clinical research, can be applied to clinical practice. Mold-active treatment is frequently and appropriately initiated on the basis of a

lower level of evidence for invasive aspergillosis than these intentionally restrictive criteria, given the potential for rapid progression of aspergillosis and death.

In patients with neutropenia, persistent fever may be the only sign of invasive fungal disease. Computed tomography (CT) of the chest is more sensitive than radiography for the detection of early pulmonary aspergillosis[58] and should be considered in patients with 10 to 14 days of neutropenia (neutrophil count, <500 per cubic millimeter) and persistent or recurrent fever of unknown cause that is unresponsive to empirical antibacterial agents.[59] The earliest radiologic sign of invasive aspergillosis is a nodule.[60] A "halo sign," defined as a macronodule surrounded by a perimeter of ground-glass opacity corresponding to alveolar hemorrhage, is suggestive of invasive aspergillosis in patients with compatible host factors. The initiation of treatment on the basis of this sign has been associated with an improved response, as compared with initiation for more advanced fungal disease.[60,61] However, other mold pathogens (e.g., zygomycetes) and bacterial pathogens capable of angioinvasion (e.g., *Pseudomonas aeruginosa*) can produce a similar appearance. Other radiographic findings that are associated with invasive aspergillosis are consolidation, wedge-shaped infarcts, and cavitation, with the latter typically occurring after neutrophil recovery (Fig. 2).

Mycologic criteria require either isolation of aspergillus species from the sinopulmonary tract or positive antigen-based laboratory markers. Cultures of bronchoalveolar lavage fluid have at best a sensitivity of 50% in focal pulmonary lesions.[62] Antigen-based diagnosis relies on serum detection of either galactomannan or beta-D-glucan, two constituents of fungal-cell walls. The galactomannan assay is relatively specific for invasive aspergillosis, whereas the beta-D-glucan assay also detects other invasive fungal diseases, including candidiasis, other mold pathogens (excluding zygomycetes), and *Pneumocystis jiroveci* (formerly called *P. carinii*).[63,64]

The serum galactomannan assay has been principally studied in patients with leukemia and recipients of hematopoietic stem-cell transplants, and its performance varies in different reports. In a meta-analysis, the serum galactomannan assay had a sensitivity of 71% and a specificity of 89%, with significant heterogeneity in diagnostic accuracy among different groups of patients.[65] The use of antifungal agents with activity against molds decreases the sensitivity of the galactomannan assay.[66] There are causes of false positive results, including concomitant use of piperacillin–tazobactam, other beta-lactam antibiotics, and gluconate-containing intravenous fluids. Cross-reactivity with other fungi (e.g., *Histoplasma capsulatum*) that have a cell-wall galactomannan similar to that of aspergillus species can cause positive results.[67]

In a patient who is at high risk for invasive aspergillosis and who has a compatible radiologic lesion (e.g., nodule or infiltrate), a positive serum galactomannan assay or culture of an aspergillus species from respiratory secretions provides strong evidence for invasive aspergillosis and can avert the need for an invasive procedure. Galactomannan detection in bronchoalveolar lavage fluid appears to be more sensitive than detection in serum[56,67] and can be used to support a diagnosis of probable aspergillosis.[57] Diagnostic testing of bronchoalveolar lavage fluid should include bacterial, fungal, and viral pathogens on the basis of the nature of the immunocompromised state and radiologic findings.[59] As an alternative to bronchoscopy, percutaneous lung biopsy may be attempted for analysis of peripheral nodules. Thoracoscopic lung biopsy should be considered in a patient whose condition is deteriorating when less invasive procedures have produced negative results. Thrombocytopenia may limit the ability to perform invasive procedures.

Diagnosis of invasive fungal diseases with the use of polymerase-chain-reaction assay, although promising, is currently investigational. Potential advantages include rapidity, low cost, and the ability to establish a diagnosis at the species level and to detect genes that confer antifungal resistance. Limitations include a lack of standardized methods, difficulty in reliably distinguishing fungal colonization from disease, and the potential for contamination with fungal DNA.[68]

THERAPY FOR INVASIVE ASPERGILLOSIS

The guidelines of the Infectious Diseases Society of America[40] recommend the use of voriconazole as the primary therapy for invasive aspergillosis. Voriconazole was more effective than amphotericin B deoxycholate as initial therapy for invasive aspergillosis and was associated with significantly

improved survival (71% vs. 58%) in a randomized trial.[61] The rate of successful outcomes was also superior among patients who received voriconazole, as compared with amphotericin B deoxycholate (53% vs. 32%). The poorest responses to antifungal therapy occurred in patients with extrapulmonary aspergillosis and in recipients of allogeneic hematopoietic stem-cell transplants.

Urgent débridement of localized aspergillosis, such as sinusitis, cutaneous disease, and osteomyelitis, should be performed. Since zygomycosis is frequently manifested in sino-orbital disease,[69] therapy with an amphotericin B formulation, which has activity against aspergillus and zygomycete species, is advised in cases of suspected invasive fungal sinusitis, pending culture and histologic results.

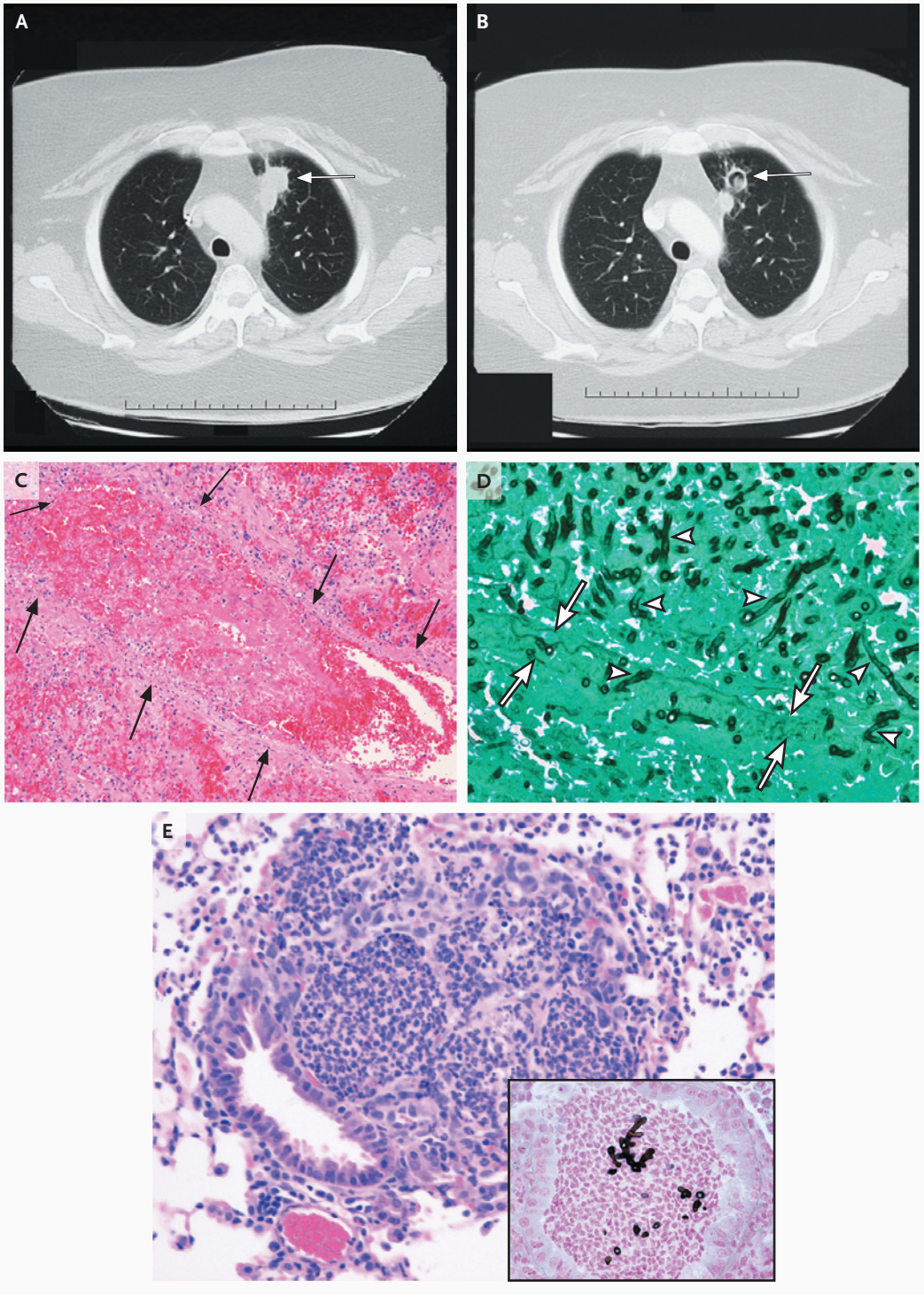
Nonlinear elimination of voriconazole in adults has important implications for dose selection. According to the package insert, it is estimated that increasing the oral dose of voriconazole from 200 mg every 12 hours to 300 mg every 12 hours leads to an increase in exposure in adults by a factor of 2.5 (area under the concentration–time curve). In contrast, clearance of voriconazole in children is linear and more rapid than in adults, which necessitates a higher dose per kilogram of body weight to achieve an exposure similar to that in adults (Table 2).[70-76] There can be considerable variability over time in voriconazole exposure, both between patients and in the same patient.[77,78] Small studies have noted a relationship between low plasma voriconazole levels and treatment failure[79,80] and between high voriconazole levels and toxicity.[80,81] Therapeutic monitoring of voriconazole should be considered in cases of refractory fungal disease or suspected drug toxicity.

When a failure in therapy is suspected, several things should be evaluated.[82] First, the diagnosis of invasive aspergillosis may be incorrect (or two infections may exist simultaneously). Second, in patients with persistent neutropenia, pulmonary lesions can increase in size, despite eventual response to antifungal therapy.[83] Third, pulmonary lesions may increase in size after neutrophil recovery because of immune reconstitution rather than failure of therapy.[84] Fourth, subtherapeutic systemic levels of mold-active azoles may occur. Finally, resistance of aspergillus species isolates to mold-active azoles is uncommon,[85] but it is also a potential cause of therapeutic failure. *A. terreus* is resistant to amphotericin B. Serial serum galactomannan assays have been used as a therapeutic marker for invasive aspergillosis, in which a falling level correlates with a successful response and a rising level with failure.[86] Such an approach is promising, but more research is required to evaluate its predictive value in different groups of patients.

There is a paucity of data to guide the administration of antifungal therapy in patients with invasive aspergillosis resistant to voriconazole; options include a lipid amphotericin B formulation, an echinocandin, and combination antifungal therapy.[40] Lipid formulations of amphotericin B are less nephrotoxic than amphotericin B deoxycholate (Table 3).[87-90] In one study, amphotericin B colloidal dispersion had a similar efficacy but less nephrotoxicity than amphotericin B deoxycholate as primary therapy for invasive aspergil-

Figure 2 (facing page). Host Factors in Radiologic and Pathologic Features of Invasive Aspergillosis.

Panel A shows a computed tomographic chest scan of a patient with neutropenia who has invasive aspergillosis in the left upper lung (arrow). A positive serum galactomannan test established the diagnosis of probable invasive aspergillosis, which averted the need for an invasive diagnostic procedure. Panel B shows cavitation of the lesion after a successful response to therapy and neutrophil recovery (arrow). Panel C shows vascular invasive aspergillosis, which is classically associated with neutropenia but can occur in patients with other conditions, such as in this case of fatal aspergillosis in a recipient of an allogeneic hematopoietic stem-cell transplant who did not have neutropenia but did have severe graft-versus-host disease. A low-power micrograph shows vascular thrombosis (with an arterial vessel outlined by arrows) that is surrounded by extensive coagulative necrosis and hemorrhage (hematoxylin and eosin). In Panel D, a high-power micrograph shows hyphae (arrowheads) transversing the blood vessel wall (outlined by arrows) and intravascular invasion (Gomori methenamine-silver, with hyphal walls staining dark). The septated hyphae, some branched at acute angles, are morphologically consistent with aspergillus species. However, other molds can have a similar appearance, so culture or molecular-based analysis at a reference laboratory is required for a definitive diagnosis. Panel E shows experimental aspergillosis in a knockout mouse model of chronic granulomatous disease, an inherited disorder of NADPH oxidase. Densely inflammatory pyogranulomatous pneumonia without vascular invasion or tissue infarction is visible (hematoxylin and eosin), with invasive hyphae in the lung as seen with silver staining (inset). These histologic features are similar to those observed in patients with chronic granulomatous disease and aspergillosis and suggest that NADPH oxidase–independent pathways are able to defend against hyphal invasion of blood-vessel walls.



losis.[90] An analysis of a large data registry on the use of amphotericin B lipid complex therapy for invasive aspergillosis showed encouraging findings regarding efficacy and safety, including the drug's tolerability in patients with renal impairment.[89] Liposomal amphotericin B that was administered at a daily dose of 3 mg per kilogram per day was associated with similar efficacy, less nephrotoxic-

Table 2. Characteristics of Mold-Active Azoles.*

Antifungal Agent	Dose†	Pharmacologic Properties‡	Comments
Voriconazole§	Intravenous therapy: 6 mg per kilogram every 12 hr for 2 doses, then 4 mg per kilogram every 12 hr as initial therapy[61]; oral therapy: for adults, 200 mg twice daily or 4 mg per kilogram twice daily[40]; for children 2 to 11 yr of age, 7 mg per kilogram twice daily without a loading dose; for children >11 yr of age, adult dose	Oral bioavailability, approximately 95%; substantial variability in systemic exposure among patients; genetic polymorphisms for CYP2C19, with 15 to 20% of Asians expected to have a slow rate of metabolism	Drug of choice as primary therapy for invasive aspergillosis (with superiority over amphotericin B in a randomized trial)[61]; consideration of therapeutic drug monitoring in select cases; poor activity against zygomycetes
Itraconazole§¶	Oral capsules: for adults, 400 mg daily (in either one or two doses); oral solution: 2.5 mg per kilogram twice daily; for children >5 yr of age: 2.5 mg per kilogram twice daily; studies in younger children are lacking; intravenous therapy: 200 mg twice daily for four doses, then 200 mg daily[40]	Oral bioavailability of the solution is better than the capsules, but both formulations have significant variability in systemic exposure; oral bioavailability of capsules (but not the solution) is increased when taken with food and is reduced by increased gastric pH	Recommended monitoring of serum trough levels aiming for >0.25 μg per milliliter on high-performance liquid chromatography[40] because of negative inotropic effects; contraindicated in patients with substantial ventricular dysfunction or a history of congestive heart failure; effective as prophylaxis in chronic granulomatous disease[70,71]; effective as therapy for corticosteroid-dependent allergic bronchopulmonary aspergillosis[72,73]
Posaconazole	Oral prophylaxis: 200 mg three times daily in patients ≥13 yr of age at high risk for invasive fungal disease[74,75]; doses of 200 mg four times daily and 400 mg twice daily have been evaluated as salvage therapy for invasive mycoses[76]; drug has not been approved by the FDA as primary or salvage therapy for invasive fungal diseases; for children 8 to 17 yr of age, a small study showed steady-state plasma levels were similar to those in adults receiving the same dose	Only available as an oral formulation, which should be taken with food or liquid nutritional supplement; a high-fat meal increases bioavailability; substantial variability in oral bioavailability among patients	Effective as prophylaxis in patients with neutropenia associated with the myelodysplastic syndrome or acute myelogenous leukemia[74] and in recipients of allogeneic hematopoietic stem-cell transplants with severe GVHD[75]; lack of studies on drug as primary therapy for invasive mycoses

* CYP2C19 denotes cytochrome P-450 2C19, FDA Food and Drug Administration, and GVHD graft-versus-host disease.
† Pediatric doses are often derived from the results of small pharmacokinetic studies, some of which are described in the package inserts or published in abstract form.
‡ The mold-active azoles are potent inhibitors of the hepatic cytochrome P-450 3A4 isoenzyme, which has a major role in the metabolism of drugs from multiple classes.
§ The intravenous formulations of itraconazole and voriconazole should be used with caution in patients with substantial renal impairment (creatinine clearance, <50 ml per minute) because of a potentially systemic accumulation of the cyclodextrin vehicle that can, in turn, cause renal toxicity. This concern does not apply to oral formulations.
¶ Intravenous itraconazole is no longer available in the United States.

ity, and a trend toward improved 12-week survival, as compared with a dose of 10 mg per kilogram daily as primary therapy for invasive aspergillosis; this study showed that increased doses of amphotericin B should not be equated with greater efficacy.[87] Pediatric patients may require a dose per kilogram of body weight that is higher than the adult dose to achieve a similar systemic exposure.[88]

Echinocandins are antifungal agents acting on the cell wall that inhibit beta-glucan synthesis (Table 4).[91-96] Caspofungin is approved by the Food and Drug Administration as salvage therapy for invasive aspergillosis. There is substantial interest in pairing echinocandins, which have cell-wall activity, with either amphotericin B formulations, which have cell-membrane activity, or mold-active azoles as therapy for invasive aspergillosis. Clin-

Table 3. Characteristics of Amphotericin B Formulations.*

Antifungal Agents	Dose	Comments
Amphotericin B deoxycholate	For invasive mold diseases: 1.0 to 1.5 mg per kilogram daily	Amphotericin B formulations are alternative options for invasive aspergillosis, such as in patients who cannot tolerate voriconazole or with refractory aspergillosis. Saline hydration (generally 1 liter for an adult of average size with normal cardiac and renal function) may prevent or ameliorate azotemia; aggressive electrolyte replacement is also required. Because of nephrotoxicity and poor tolerance of long-term use in patients with invasive aspergillosis,[61] a lipid formulation should be considered. An amphotericin B formulation should be used for suspected or proven zygomycosis.
Liposomal amphotericin B	3 to 5 mg per kilogram daily; clearance and volume of distribution are influenced by body weight in children with cancer; higher weight-based doses may be optimal in patients weighing less than 20 kg[87]	One randomized trial showed that 3 mg per kilogram per day was as effective as but less toxic than 10 mg per kilogram per day as initial therapy for invasive aspergillosis.[88]
Amphotericin B lipid complex	5 mg per kilogram daily	This drug was evaluated in a large registry database of invasive aspergillosis.[89]
Amphotericin B colloidal dispersion	5 to 6 mg per kilogram daily	This drug (at a dose of 6 mg per kilogram) had a similar efficacy, lower nephrotoxicity, and greater infusional toxicity than daily amphotericin B deoxycholate (at a dose of 1 to 1.5 mg per kilogram) as primary therapy for invasive aspergillosis.[90]

* The suggested doses of amphotericin B formulations are based on those used in clinical trials and in the guidelines of the Infectious Diseases Society of America.[40] The lipid formulations of amphotericin B have different pharmacokinetic properties, but the clinical significance of these differences is unknown.

ical data on combination antifungal therapy for invasive aspergillosis are limited but encouraging. In one provocative study, Marr et al.[97] reported a survival advantage of voriconazole plus caspofungin, as compared with voriconazole alone, in a retrospective analysis of salvage therapy for invasive aspergillosis. However, this database involved a small number of patients, and the two groups were noncontemporaneous; therefore, other host and infection-related factors may have influenced the outcome. A noncomparative study of caspofungin in combination with other antifungal agents as salvage therapy in patients with invasive aspergillosis resulted in a successful outcome in 25 of 51 patients (49%),[91] a success rate similar to that in a previous study of caspofungin monotherapy.[98] A randomized trial comparing voriconazole with voriconazole plus anidulafungin (an echinocandin) has begun.

An important component in therapy for invasive aspergillosis involves anticipating and managing the toxic effects of various drugs. Azoles cross-react with and can be inhibitors and substrates of mammalian cytochrome P-450 isoenzymes. Inhibition of the CYP3A4 isoenzyme by azoles (especially mold-active azoles, as compared with fluconazole) accounts for the majority of drug interactions. Indeed, several agents that are used to treat cancer (e.g., cyclophosphamide and vinca alkaloids) and as immunosuppressive therapy in transplant recipients (e.g., calcineurin inhibitors and sirolimus) are metabolized through hepatic CYP3A4. All azoles can cause hepatotoxicity, including hyperbilirubinemia and liver-enzyme abnormalities, both of which are usually reversible. Voriconazole commonly causes reversible visual symptoms that uncommonly require drug cessation. The main toxic effects that are associated with amphotericin B formulations are infusional reactions and nephrotoxicity. Echinocandins are generally well tolerated.

The optimal duration of therapy is not straightforward and depends on the burden of disease and host immunocompetence. The guidelines of the Infectious Diseases Society of America recommend a duration of therapy of at least 6 to 12 weeks for pulmonary aspergillosis.[40] In immunocompromised patients, therapy should be continued throughout the period of immunosuppression and until the resolution of lesions.[40] In patients with previously diagnosed invasive aspergillosis, antifungal therapy should be continued or reinitiated during subsequent periods of immunosuppression (e.g., additional cytotoxic

Table 4. Characteristics of Echinocandins.

Antifungal Agent	Dose	Pharmacologic Properties	Comments
Caspofungin	Adult dose: 70 mg daily for the first dose, then 50 mg daily; 70 mg daily can be considered for invasive aspergillosis[91]; in patients with moderate hepatic insufficiency (Child–Pugh score, 7 to 9), a first dose of 70 mg, then 35 mg daily for patients with moderate liver disease; pediatric dose: 50 mg per square meter of body-surface area (35 mg per square meter in patients with moderate liver disease)[92]	Slowly metabolized by hydrolysis and N-acetylation and undergoes spontaneous degradation	Only echinocandin that is FDA-approved as salvage therapy for invasive aspergillosis; encouraging results from a preliminary, nonrandomized database regarding combination voriconazole and caspofungin as therapy for invasive aspergillosis
Micafungin	Prophylaxis in recipients of stem-cell transplant recipients during neutropenia: 50 mg daily[93]; for candidemia and other forms of invasive candidiasis, 100 mg daily; for esophageal candidiasis, 150 mg daily; optimal dose for invasive aspergillosis has not been defined; pediatric dose: increased clearance as a function of decreasing age occurs[94]	Hepatically metabolized by arylsulfatase and catechol-*O*-methyltransferase; although a substrate of CYP3A4 in vitro, CYP3A4 does not play a significant role in metabolism in vivo	A nonrandomized trial showed safety alone and paired with other agents as primary and salvage therapy for invasive aspergillosis[95]
Anidulafungin	For candidemia and other forms of invasive candidiasis: 200 mg for first dose, then 100 mg daily; optimal dose for invasive aspergillosis has not been defined; pediatric dose: 0.75 mg and 1.5 mg per kilogram daily resulted in drug exposure similar to that of adult doses of 50 and 100 mg daily, respectively[96]	Undergoes slow chemical degradation; not hepatically metabolized	No clinical trial data are available regarding therapy for invasive aspergillosis, although a randomized trial is under way comparing voriconazole plus anidulafungin with voriconazole alone as primary therapy for invasive aspergillosis

chemotherapy or hematopoietic stem-cell transplantation) to prevent recrudescence.[40,99]

When feasible, immunosuppressive therapy (e.g., corticosteroids) should be reduced or discontinued. Adjunctive myeloid colony-stimulating factors (granulocyte or granulocyte–macrophage colony-stimulating factor) should be considered in patients with neutropenia who have severe infections, such as aspergillosis.[100] Treatment with granulocyte transfusions in patients with persistent neutropenia and the use of recombinant interferon-γ (which activates neutrophils and macrophages) can be considered in patients who have refractory or disseminated aspergillosis; however, the benefit of such therapies versus their toxic effects has not been established.[25]

PREVENTION OF INVASIVE ASPERGILLOSIS

Prevention of invasive aspergillosis relies on environmental infection-control guidelines to reduce mold exposure[101] and antifungal prophylaxis targeted to high-risk patients. Mold-active azoles, echinocandins, and amphotericin B formulations are options.[40,59] Prophylactic inhalation of amphotericin B formulations, which may prevent systemic toxic effects, merits further evaluation. Posaconazole was effective as prophylaxis in patients with neutropenia who had the myelodysplastic syndrome and acute myelogenous leukemia[74] and in patients with severe GVHD.[75]

Limitations to mold-active prophylaxis exist. Mold-active azoles can cause serious drug interactions. The diagnosis of breakthrough aspergillosis may be hampered by false negative results on serum galactomannan assays. There is also a gap in knowledge regarding optimal therapy for breakthrough invasive aspergillosis in patients receiving mold-active prophylaxis; changing the antifungal class is a reasonable, although untested, option. There is debate about the overall benefit, risks, and cost-effectiveness of mold-active prophylaxis in high-risk patients as compared with a

preemptive approach, in which a mold-active agent is targeted to patients who meet prespecified criteria on the basis of radiologic findings, laboratory markers, or both.[102,103]

FUTURE PERSPECTIVES

The development of new diagnostic and therapeutic approaches will be facilitated by knowledge of both host and pathogen characteristics that predispose patients to aspergillus-associated diseases. Knowledge of innate and T-cell immunity against aspergillus may pave the way to new immunomodulation strategies, including vaccine development.[25] In addition, antifungal agents have immunomodulatory effects, including the activation of pathogen-recognition receptors and unmasking of proinflammatory constituents of fungal-cell walls that may be clinically relevant and therapeutically exploitable.[104,105]

Polymorphisms in host genes that mediate innate immunity may influence the risk of invasive aspergillosis during periods of immunosuppression. As examples, Bochud et al.[23] found that specific donor TLR4 haplotypes influenced the risk of invasive aspergillosis in recipients of allogeneic hematopoietic stem-cell transplants. Zaas et al.[106] found that polymorphisms in the plasminogen allele affected the outcome of experimental aspergillosis in mice and the risk of invasive aspergillosis in recipients of allogeneic hematopoietic stem-cell transplants. Alterations in plasminogen can affect fungal virulence in a number of ways, including fungal adherence to extracellular matrix and a direct effect on innate immunity. Knowledge of these host genetic factors may prove to be important in stratifying the risk of invasive aspergillosis during periods of immunosuppression, in guiding donor selection and targeted antifungal prophylaxis, and in identifying new therapeutic targets.

An increased understanding of fungal genetics and biochemistry has led to therapeutic strategies at the preclinical level, such as inhibition of cell stress-response pathways.[107,108] The recent discovery that *A. fumigatus* has a sexual reproductive cycle provides insight into its evolution and genomic variability and offers a valuable tool to analyze the genetic basis of pathogenicity.[109] In addition, genomic analysis has shown that aspergillus species are extremely diverse.[110] One potential benefit of comparative genomics among pathogenic and nonpathogenic aspergillus species is to identify genes associated with virulence that can be targets for drug development.

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