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Innate Immunity to *Aspergillus* Species

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INTRODUCTION

Aspergillus species are among the most common molds encountered by humans and are the etiologic agents for a remarkably diverse set of human diseases. With the exception of diseases caused by *Aspergillus*-derived mycotoxins, the host's response during its encounter with this microorganism is the key determinant in whether the host clears the microorganism without developing disease or whether the host is colonized by the microorganism, is infected by it, or develops a hypersensitivity illness as a result of the encounter. Despite constant exposure to *Aspergillus* conidia, it is remarkable that most humans do not develop any illness attributable to these organisms and have no evidence of antibody- or cell-mediated acquired immunity to this organism (58, 99). This suggests that, for most healthy humans, innate immunity is sufficient to clear the organism before acquired immunity is called upon. In this review, we provide an outline of the early events in the host's immune response to *Aspergillus* species.

The usual niche of *Aspergillus* species is in soil and decaying biomass. *Aspergillus* species grow as multicellular branching hyphae and reproduce asexually by means of aerial conidiophores; most *Aspergillus* species do not have a recognized teleomorph (sexual form). The reproductive spores, known as conidia, are produced in very large numbers and, by virtue of their small size and hydrophobic exterior, remain airborne for hours once released. Resting conidia are metabolically quiescent and can remain viable for months. The development of new colonies begins with swelling of the conidia within hours of arriving in a permissive environment and is followed by the germination and subsequent elongation of hyphae.

The mean concentration of *Aspergillus* conidia in air is 0.2 to 15 conidia/m^3 according to different studies and is up to 10^6 conidia/m^3 in some agricultural settings (165). As a result, humans routinely inhale hundreds of conidia daily. Despite this constant exposure, it is remarkable that most humans do not develop any illness attributable to these organisms. On the other hand, most human diseases caused by *Aspergillus* species begin in the respiratory tract. Among the >180 recognized *Aspergillus* species, *A. fumigatus* remains the most common cause of human disease; however, other species including *A. flavus*, *A. niger*, *A. nidulans*, and *A. terreus* are increasingly frequent causes of disease (67). The human diseases caused by these organisms are extraordinarily diverse and have been considered under three categories (Table 1): invasive infections, which are characterized by the growth of hyphae within tissues; infections caused by the colonization of mucosal surfaces without invasion into tissue; and hypersensitivity diseases, which are defined as diseases caused by the immune response of the host. Thus, while in the vast majority of healthy hosts the microorganism is cleared without causing any disease, the encounter between *Aspergillus* and the host can result in a broad

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TABLE 1. Categories of human diseases caused by *Aspergillus* species

Category	Example(s) of specific diseases	Defect in host defense	Animal model
No disease		Healthy host	Intrapulmonary challenge of wild-type mice with conidia
Invasive infection	Invasive pulmonary aspergillosis, invasive rhinosinusitis, invasive tracheobronchial aspergillosis, and chronic cavitary pulmonary aspergillosis	Impaired cell-mediated immunity (including cell-mediated innate immunity)	Intrapulmonary challenge of immunocompromised animals with conidia (antibody-mediated neutrophil depletion, chemotherapeutic drugs, corticosteroids)
Colonization	Pulmonary mycetoma in preexisting lung cavities, asymptomatic (e.g., in bronchiectasis, chronic obstructive pulmonary disease)	Impaired mucosal immunity	No established model to date
Hypersensitivity	Asthma, allergic bronchopulmonary aspergillosis, allergic sinusitis, and hypersensitivity pneumonitis (e.g., malt worker's lung)	Misdirected acquired immunity	Intrapulmonary challenge of sensitized mice with conidia

range of diseases. Consistent with the damage-response framework of microbial pathogenesis (32, 33), colonization is distinct from commensalism and describes a form of infection that can result in continued low-level damage to the host and not a state of benign coexistence. The key determinant of the pathogenicity of *Aspergillus* species, and the reason for the diversity of host outcomes, is hypothesized to be the nature of the immune response of the host (32, 33), and the diseases may be conceptualized as points along a spectrum of abnormal immune responses of the host (Fig. 1).

Overview of Immunity to *Aspergillus*

From an immunological perspective, the defense against inhaled conidia begins in the physical barriers of the respiratory tract (Fig. 2). These include the nasal turbinates and the branching pattern of the bronchial tree, which results in a highly turbulent airflow that deposits most inhaled particles against the airway surface fluid, allowing for their removal by the ciliary action of the respiratory epithelium. This constitutes a major mechanism of antimicrobial defense in the lungs (83).

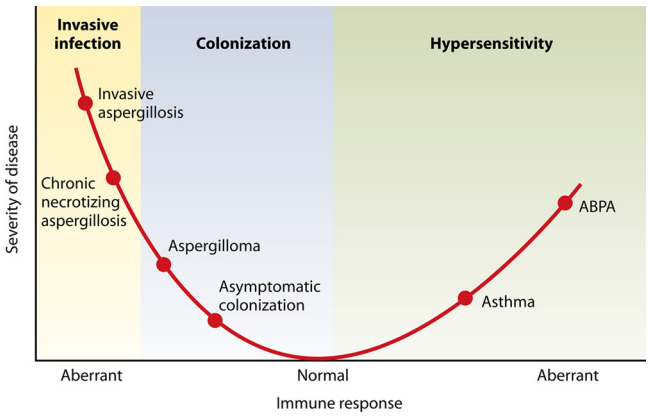

FIG. 1. Diagrammatic representation of diseases attributed to *Aspergillus* species as a function of the host's immune response. ABPA, allergic bronchopulmonary aspergillosis.

On the other hand, the small size of resting *Aspergillus* conidia (2 to 5 μm in diameter) allows some of the inhaled spores to avoid this defense mechanism and arrive in the respiratory zone of the lung, beyond the ciliated epithelium. Not only is the airway lining a passive means of trapping inhaled particulates in mucus, but it also contains a rich array of soluble pathogen recognition receptors and microbicidal peptides. The recognition of *Aspergillus* species by the host is achieved by means of these soluble pattern recognition molecules as well as cell-bound receptors. The next step in defense against *Aspergillus* species is the activation of the effector mechanisms of innate immunity; these include the antimicrobial mechanisms of resident lung leukocytes such as alveolar macrophages and dendritic cells, recruitment of other leukocytes, and activation of recruited leukocytes after their arrival at the site of infection. Coincident with this, resting conidia become swollen within 4 to 5 h of arrival in the lungs and, if not cleared, germinate and form hyphae within 12 to 15 h of arrival. The hyphal forms invade the adjacent lung tissue, causing pneumonia, and often disseminate to other organs, most commonly the contralateral lung and the brain. If the organism has not been cleared, antigen presentation and clonal proliferation of *Aspergillus*-specific T-cell clones over the ensuing days result in the initiation of acquired immunity against the organism.

A key concept in the study of immune responses to *Aspergillus* is that the susceptibilities of the host determine the morphological form, antigenic structure, and physical location of the fungus. In healthy hosts inhaling small numbers of conidia (a circumstance encountered by all humans every day), conidia are successfully cleared by epithelial mucociliary defense mechanisms, and the occasional conidia reaching the alveoli may be dealt with by resident phagocytes without an initiation of the recruitment cascade. In a host with impaired mucosal defenses, such as patients with bronchiectasis or preexisting lung cavities (which are lined with metaplastic epithelial cells), the conidia germinate and form hyphae on the luminal side of the abnormal mucosal surface and initiate a robust inflammatory response centered on the airway. In contrast, invasive infection, by definition, involves the inva-

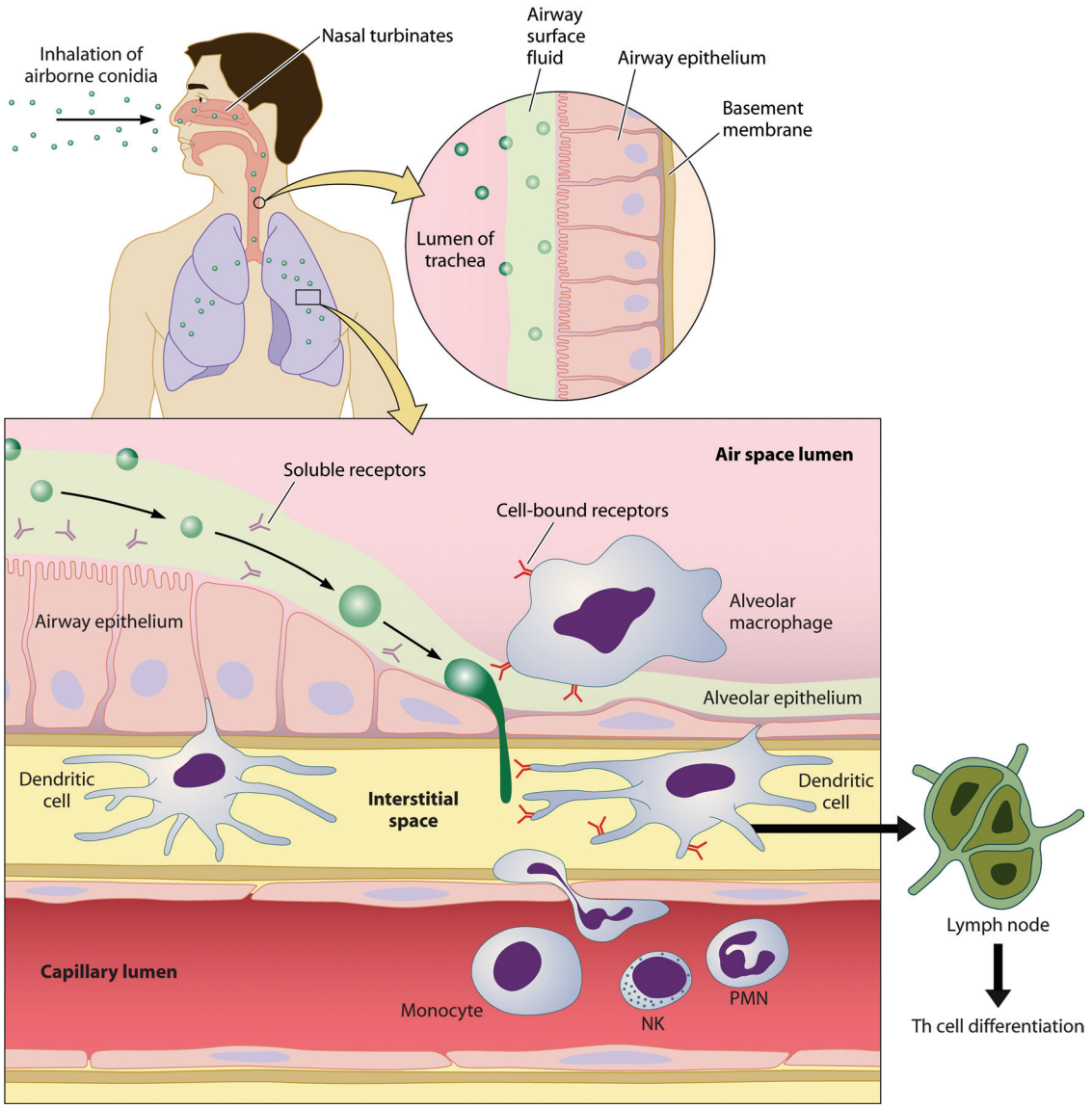

FIG. 2. Schematic representation of components of the host response to inhaled *Aspergillus* conidia. PMN, polymorphonuclear leukocytes.

sion of the hyphae into the lung parenchyma. The most clearly defined predisposing factor is a quantitative or qualitative defect in neutrophils, but the increasing number of nonneutropenic immunocompromised patients with invasive aspergillosis underlines the importance of nonneutrophil defense mechanisms in preventing this infection: these mechanisms may include the recognition of the microorganism, recruitment of leukocytes other than neutrophils, and effector mechanisms of recruited or resident cells. The mechanism of immunosuppression is an important consideration in interpreting experimental data from animal models of invasive aspergillosis: animals treated with a neutrophil-depleting antibody develop neutropenia without obvious effects on other leukocyte populations, whereas mice treated with cyclophosphamide are depleted of multiple lymphocyte subsets in addition to neutrophils, and animals treated with corticosteroids have normal numbers of neutrophils but multiple defects in antimicrobial immune responses (8, 125, 159). In hypersensitivity diseases, aberrant acquired immune responses are directed at *Aspergillus* antigens contained in, or elaborated by, conidia (for example, in a subset of patients with asthma and hypersensitivity pneumonitis) or colonizing hyphae (for example, in allergic bronchopulmonary aspergillosis).

RECOGNITION OF *ASPERGILLUS* SPECIES BY THE HOST

The recognition of *Aspergillus* conidia and hyphae occurs via a number of soluble and cell-associated microbial pattern recognition receptors. Conidial maturation triggers a profound morphological change that involves the loss of the proteinaceous hydrophobic layer and exposure of the inner cell wall (120, 163). This cell wall is composed mainly of polysaccharides consisting of β-glucan, mannan, chitin, and galactomannan (89). The morphological state of *Aspergillus* is critical to its

recognition by the host: the binding and ingestion of resting conidia, for example, induce very little inflammatory response (64, 74, 157), and optimal $CD4^+$ T-cell responses appear to occur only in response to live conidia (136). Antifungal drugs that target and modulate fungal wall components have also been shown to alter inflammatory responses: in vitro studies show that the targeting of the synthesis of β-(1,3)-glucan with echinocandins results in increased β-glucan exposure at the tips of *Aspergillus* hyphae that is associated with higher levels of tumor necrosis factor (TNF) and CXCL2 secretion by bone marrow-derived macrophages (73) and increased neutrophil-mediated hyphal damage (86).

Soluble Receptors

Pulmonary collectins are a family of C-type lectins that include lung surfactant proteins A and D and mannan-binding lectin. These soluble receptors serve as opsonins for many microorganisms and have been shown to bind *A. fumigatus* conidial carbohydrate structures in a calcium-dependent manner (3, 95, 97, 118). Surfactant proteins A and D are essential for both normal lung function and host defense (102, 131, 173). Surfactant proteins A and D promote the agglutination of conidia and their binding to neutrophils and alveolar macrophages and enhance the phagocytosis and killing of conidia by neutrophils (95). Study of the in vivo role of these receptors in animal models has been complicated by the baseline derangement of alveolar macrophages and type II alveolar epithelial cells observed in surfactant protein A and especially surfactant protein D gene knockout animals (90). However, the administration of exogenous surfactant protein D to wild-type mice provides protection in a corticosteroid-induced model of invasive aspergillosis (97). Patients with allergic bronchopulmonary aspergillosis have higher serum concentrations of surfactant protein D, and in a mouse model of pulmonary hypersensitivity to *Aspergillus* species, there was a parallel marked induction in the expression of surfactant protein D (but not surfactant protein A) in the lungs that was mediated by interleukin-4 (IL-4) and IL-13 (68, 69, 85). Finally, the administration of exogenous surfactant proteins A and D to mice with pulmonary hypersensitivity to *Aspergillus* results in an attenuated obstructive defect, airway pathology, Th2 cytokines, and lung histamine release (55, 96).

There is substantial evidence for an involvement of several components of the complement cascade in response to *Aspergillus*. The binding of C3 to *A. fumigatus* conidia and hyphae led to the activation of the complement alternative pathway (84, 160). In contrast, mannan-binding lectin promotes the activation of the lectin complement pathway via C4bC2a (78) and results in a dose-dependent deposition of complement on conidia and hyphae (54, 78, 84, 118, 160). The incubation of *Aspergillus* conidia with healthy human serum also activates the alternative pathway via the mannan-binding lectin C2 bypass mechanism (54). Complement activation may be influenced by the antigenic structure of *A. fumigatus* strains, as clinical strains isolated from patients with invasive aspergillosis induced a stronger activation of the alternative pathway than did environmental strains (54). In the context of in vivo animal models, mannan-binding lectin is not necessary for antifungal defense in immunocompetent hosts, since mannan-binding lectin gene knockout mice are not susceptible to invasive aspergillosis (72). However, the administration of exogenous mannan-binding lectin to corticosteroid-treated mice with invasive aspergillosis resulted in improved survival and reduced lung fungal burden in infected mice. This improved outcome was associated with enhanced production of TNF and gamma interferon (IFN-γ) and reduced production of IL-10 by cultured splenocytes of infected animals (78). On the other hand, resting *Aspergillus* species are capable of binding several complement regulatory proteins including factor H and plasminogen, thereby inhibiting the activation of the complement cascade (13). In this context, a mutated form of the plasminogen gene was associated with susceptibility to invasive aspergillosis in immunocompromised mice and a similar single-nucleotide polymorphism in human plasminogen predisposes hematopoietic stem cell recipients to invasive aspergillosis (174).

Pentraxin-3 belongs to the family of long pentraxins and is secreted as a multimeric protein by a variety of cells in response to inflammatory mediators (4, 25, 51, 70, 145). The presence of conidia can rapidly promote the production of pentraxin-3 in mononuclear phagocytes and dendritic cells (62). This soluble receptor binds galactomannan on *Aspergillus* conidia and facilitates recognition by effector cells. The critical role of pentraxin-3 in host antifungal defense was demonstrated in a series of in vitro and in vivo experiments (62, 77). In vitro, pentraxin-3-deficient alveolar macrophages and neutrophils had reduced phagocytic and conidiocidal activities, and pentraxin-3-deficient dendritic cells had defective IL-12 production and upregulation of major histocompatibility complex class II and CD86 in response to the fungus, whereas the addition of exogenous pentraxin-3 restored the antifungal effector activities and responses to *Aspergillus* conidia in gene-deficient cells. In vivo, otherwise immunocompetent pentraxin-3-deficient mice were highly susceptible to invasive aspergillosis. This was associated with a concomitant increase in fungal load and IL-4 levels but a decrease in IFN-γ levels in the lungs. Both the systemic and local administration of exogenous pentraxin-3 resulted in improved outcomes for these animals. In addition, the adoptive transfer of wild-type neutrophils was sufficient to decrease fungal growth in pentraxin-3-deficient animals challenged with *A. fumigatus* (77).

Cell-Bound Receptors

Mammalian Toll-like receptors (TLRs) are a family of nine structurally conserved receptors that recognize and mediate cellular responses to conserved pathogen-associated molecular patterns. The adaptor molecule MyD88 is a major (but not exclusive) signaling mechanism of the TLRs that induce the production of an array of inflammatory cytokines and reactive oxygen species. A number of studies have examined the role of specific TLRs in mediating the recognition of *A. fumigatus*.

Several in vitro studies have examined the role of TLR2 and TLR4 in the detection of *Aspergillus* species by leukocytes (10, 14, 15, 53, 98, 100, 107, 117, 157, 168). While those studies may appear to yield conflicting results at first glance, a detailed comparison of the experimental approaches shows that the discordant results are likely related to the use of different host cells, different *Aspergillus* morphotypes, and different measures of the host response (Table 2).

TABLE 2. In vitro studies of the role of TLR2 and TLR4 in the response of primary leukocytes to *A. fumigatus*[a]

Host cell	*Aspergillus* morphotype	Effect(s)	Reference
Human adherent PBMC (monocytes)	Ethanol-killed serum-opsonized hyphae	TLR4 but not TLR2 required for TNF response	168
Human PBMC	Heat-killed conidia and hyphae (nonopsonized)	TLR2 but not TLR4 required for TNF response to both conidia and hyphae	117
Mouse-resident peritoneal macrophages	Heat-killed conidia and hyphae (nonopsonized)	TLR4 required for TNF and IL-1α/β response to conidia but not hyphae TLR2 required for TNF and IL-1α/β response to both conidia and hyphae TLR2 required for IL-10 response to hyphae but not conidia	
Mouse-elicited peritoneal macrophages	Live resting conidia, heat-killed swollen conidia, and hyphae (nonopsonized)	MyD88 required for TNF response to all TLR2 required for TNF response to resting conidia and hyphae CD14 not required	98
Mouse elicited peritoneal macrophages	Ethanol-killed conidia and hyphae (nonopsonized)	TLR2 and TLR4 required for TNF response to conidia and hyphae and MIP-2/CXCL2 response to hyphae	107
Mouse bone marrow-derived macrophages	Heat-killed conidia and hyphae (nonopsonized)	MyD88 not required for phagocytosis or killing of conidia or TNF response to hyphae	100
Mouse alveolar macrophages	Conidia (nonopsonized)	TLR2 required for TNF response	10
Mouse alveolar macrophages	Heat-killed conidia (nonopsonized)	TLR2 required for TNF response but not CCL3/MIP-1α, CXCL2/MIP-2, IL-1α/β, IL-6, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor responses	157
Mouse alveolar macrophages	Conidia (nonopsonized)	TLR2, TLR4, or MyD88 not required for phosphorylation of ERK or p38 mitogen-activated protein kinases	53
Mouse-elicited peritoneal neutrophils	Conidia and hyphae (nonopsonized)	TLR2 required for conidial but not hyphal killing TLR4 required for conidial and hyphal killing	15
Mouse lung dendritic cells	Conidia with amphotericin (nonopsonized)	TLR2 absence resulted in greater IL-12p70 and reduced IL-10 production levels TLR4 or MyD88 absence resulted in reduced IL-12p70 and greater IL-10 production levels	14

[a] PMBC, peripheral blood mononuclear cells.

In the in vivo setting, otherwise immunocompetent mice that are genetically deficient in TLR2, TLR4, IL-1R1, or MyD88 are not susceptible to invasive aspergillosis when challenged with conidia via the respiratory tract (14, 23, 53): these mice display lung histologies and cytokine production that are comparable to those of wild-type mice. Nevertheless, TLR signaling via MyD88 appears to be necessary for the early inflammatory responses to *Aspergillus* species in immunocompetent hosts (24): in the absence of MyD88, there were fewer natural killer (NK) cells and higher fungal burdens in the infected lung within 24 h of fungal challenge. In addition, MyD88-mediated signaling was important for the subsequent development of protective adaptive responses (14, 135). In contrast to immunocompetent animals, mice with cyclophosphamide-induced immunosuppression require TLR4 and MyD88 for optimal host defense against invasive aspergillosis (14): TLR4 and MyD88 deficiencies each led to significantly lower survival rates, higher lung fungal contents, higher numbers of IL-4-producing but lower numbers of IFN-γ-producing CD4 T cells in thoracic lymph nodes, and, in MyD88-deficient hosts, reduced lung TNF levels (14).

The consequences of the absence of TLR2 in in vivo infection are more complex: in cyclophosphamide-treated mice, a TLR2 deficiency did not influence survival but resulted in an increased lung fungal content. This was associated with higher numbers of lung IL-4-producing CD4 T cells in thoracic lymph nodes but also with higher lung TNF levels (14). These findings are in contrast to findings using a model of invasive aspergillosis in mice immunosuppressed with vinblastine, in which *Tlr2*$^{-/-}$ animals had higher mortality rates and significantly lower levels of lung TNF than did wild-type mice (10).

TLR9 can initiate immune responses to *Aspergillus* species via the recognition of fungal unmethylated CpG DNA in murine bone marrow-derived dendritic cells and human

plasmacytoid dendritic cells (130). Surprisingly, TLR9-deficient mouse-elicited peritoneal neutrophils have a greater ability to kill *Aspergillus* conidia and hyphae (15), and TLR9-deficient mouse lung dendritic cells produce less IL-12p70 and more IL-10 in response to conidia (14). However, the role of TLR9 in the context of in vivo defense against *Aspergillus* species appears to be complex: in the setting of immunosuppression with cyclophosphamide or antibody-mediated neutrophil depletion, TLR9-deficient mice survive longer and have significantly lower fungal burdens than wild-type mice following challenges with *A. fumigatus* conidia (14, 15, 129), suggesting the involvement of TLR9 signaling in an immunoregulatory mechanism that ultimately benefits *Aspergillus* species and may be mediated by neutrophils. In the context of a model of airway hypersensitivity to *Aspergillus*, however, the absence of TLR9 led to lower levels of methacholine-induced airway hyperreactivity but promoted fungal growth in the lung associated with reduced lung dectin-1 expression levels; this is remarkable since wild-type mice sensitized to *Aspergillus* species do not develop invasive diseases following the administration of even large inocula in the setting of neutrophil depletion (71, 122). It remains to be established whether this effect is due to a failure of TLR9-deficient mice to develop acquired immunity to *Aspergillus* species during the sensitization protocol or whether this finding is due to the absence of a TLR9-mediated recognition of *Aspergillus* species during secondary challenge with intratracheal conidia.

A negative regulator of TLR-receptor signaling, Toll IL-1R8 (alternative name, immunoglobulin IL-1-related receptor), has been studied using immunocompetent mice challenged with intrapulmonary conidia. The absence of Toll IL-1R8 resulted in reduced survival rates and increased levels of lung fungal growth that were associated with elevated lung IL-17 and IFN-γ levels but lower IL-10 and Foxp3 transcript levels a week after infection, suggesting that the absence of this regulatory process results in the detrimental activation of Th1 and Th17 immunity (23). Another group of cell-bound G-protein-coupled cell surface receptors, the protease-activated receptors (PARs), have recently been shown to influence in vivo responses to *Aspergillus* species (112). Cyclophosphamide-treated mice deficient in PAR_2 or treated with a PAR_2 antagonist displayed higher lung oxidative burst and MMP-9 activities, higher lung TNF protein levels, and lower lung IL-10 levels after challenge with *Aspergillus* conidia (112). Consistent with this, the transgenic expression of PAR_2 and treatment with a PAR_2 agonist had the reverse effects (112), suggesting that PAR_2 signaling attenuates responses in the context of invasive aspergillosis.

Several polymorphisms of human TLRs have been associated with an increased risk of invasive aspergillosis in susceptible hosts. A haplotype of TLR4, which consists of two single-nucleotide polymorphisms within the coding region of the gene that are associated with hyporesponsiveness to lipopolysaccharide, resulted in a hazard ratio of 2 to 4 for invasive aspergillosis in allogeneic hematopoietic stem cell transplant recipients when the polymorphism was present in the donor (18). This polymorphism was also associated with an increased risk of chronic necrotizing aspergillosis in a separate cohort (31). Another study linked polymorphisms in TLR1 and TLR6 in recipients of allogeneic hematopoietic stem cell transplantation to risk of invasive aspergillosis (79), and a polymorphism in TLR9 was associated with allergic bronchopulmonary aspergillosis (31). These observations provide indirect evidence of the relevance of TLRs in human aspergillosis.

Dectin-1 is a C-type lectin-like receptor that was initially identified as being a dendritic cell receptor (6, 27). Dectin-1 is a major receptor for fungal β-glucans (28) and is widely expressed in myeloid leukocytes including macrophages, neutrophils, and dendritic cells (29, 108, 162). Studies have demonstrated a stage-specific activation of dectin-1 in response to *Aspergillus* species and provide a mechanism in which the host inflammatory response is triggered only in the presence of swollen conidia (64, 74, 94, 157). The protective role of dectin-1 was demonstrated using immunocompetent mice challenged with *A. fumigatus*, in which a blockade of dectin-1 reduced production of inflammatory cytokines and increased lung fungal burden (157, 172). In an immunosuppressed model of invasive aspergillosis, the administration of a synthetic dectin-1–Fc receptor fusion protein resulted in delayed mortality through a mechanism that involved enhanced conidial killing by alveolar macrophages (101). In immunocompetent mice, this enhanced susceptibility was associated with reduced lung neutrophil accumulation and a failure to induce the expression of IL-23 and IL-17 in the lungs in the first 24 h after infection (172).

Dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) is another C-type lectin present on surfaces of dendritic cells and some macrophages with specificity for high mannose moieties (57, 127). Human lung dendritic cells and alveolar macrophages bind *Aspergillus* conidia via the interaction of DC-SIGN with fungal galactomannan (154). While the binding and ingestion of conidia appear to be influenced by the cell surface expression of DC-SIGN, the precise contribution of this interaction to the host antifungal response is yet to be fully elucidated.

Cytokine Signaling of Recognition

Pathogen recognition via soluble and cell-bound microbial pattern recognition receptors is quickly followed by the afferent limb of pathogen recognition, which consists of the elaboration of an initial group of cytokines including TNF and members of the IL-1 family. Among members of the IL-1 family, IL-1β is induced in alveolar macrophages in response to *Aspergillus* antigens and in peripheral blood monocytes in response to *Aspergillus* conidia and hyphae in in vitro studies (119, 170). In animal models, IL-1β is induced in mice with chronic glaucomatous disease and invasive aspergillosis, and IL-18 is induced in the lungs of immunocompetent mice and sensitized mice challenged with intrapulmonary conidia (16, 26, 113). The precise mechanism of action of these ligands in host defense against *Aspergillus* species has not been evaluated in detail: although the neutralization of IL-18 alone did not affect lung fungal killing in immunocompetent mice, the neutralization of both IL-18 and TNF did result in greater lung fungal viability (26). In mice with airway allergy to *Aspergillus*, however, the immunoneutralization of IL-18 has been shown to result in a prolonged retention of *Aspergillus* in the airways, lower TLR2 expression levels, and greater airway remodeling (16), suggesting that this innate immune mechanism is relevant

to pathology in the context of acquired hypersensitivity responses to *Aspergillus*.

TNF is a 17-kDa protein that is secreted predominantly by cells of myeloid lineage, including alveolar macrophages, dendritic cells, recruited monocytes/macrophages, and neutrophils. TNF is markedly induced when cells of the monocyte/macrophage lineage are coincubated with *Aspergillus* antigens or fungal elements and is markedly induced in the lungs of both immunocompetent and immunocompromised mice after intrapulmonary challenge with conidia (26, 105, 150). In both immunocompetent animals and immunocompromised mice treated with cyclophosphamide, immunoneutralization of TNF results in an impaired fungal clearance and increased mortality that were associated with lower lung levels of several chemokines (CXCL1/KC, CXCL2/macrophage inflammatory protein 2 [MIP-2], CCL2/monocyte chemoattractant protein 1 [MCP-1], and CCL3/MIP-1α) and lower levels of recruitment of neutrophils to the lungs (26, 104). Conversely, the pretreatment of immunocompromised animals with a TNF agonist resulted in markedly attenuated infection (105). The importance of TNF in the defense against *Aspergillus* in humans has since been supported by documentation that otherwise immunocompetent patients treated with TNF antagonists are susceptible to invasive aspergillosis (146, 164, 169).

RECRUITMENT OF LEUKOCYTES TO SITE OF INFECTION

Leukocyte recruitment is a complex and multistep process, which begins with the interaction of circulating leukocytes and endothelial surface adhesion molecules, leading to the rolling and adherence of leukocytes, followed by the extravasation of the leukocytes into the extravascular space and finally directional homing to the site of inflammation. Among classes of molecules involved in these processes, *Aspergillus* hyphae have been shown to induce endothelial cells to generate E-selectin and VCAM-1 both in vitro and in models of invasive aspergillosis in mice treated with cyclophosphamide and also mice treated with corticosteroids (39).

Among the many classes of mediators involved in this process, several chemokine ligands and receptors have been examined in the context of innate defenses against *Aspergillus* species. Chemokine ligands are a superfamily of 8- to 14-kDa structurally related peptides that are divided into CC, CXC, C, and CX_3C families based on the sequence of cysteine residues near the amino terminus. The chemokine receptors belong to the family of seven-transmembrane G-protein-coupled transmembrane molecules. Unlike most cytokines, which are the products of primarily leukocytes, diverse cell types are capable of producing chemokines. In response of *Aspergillus* conidia and hyphae, for example, macrophages, dendritic cells, alveolar and bronchial epithelial cells, and endothelial cells have been shown to generate these ligands (39, 46, 60, 73, 74, 108, 128, 156).

Recruitment of Neutrophils

A subset of the CXC family of chemokine ligands is defined by the presence of a glutamic acid-leucine-arginine (ELR) motif immediately downstream of the CXC sequence. These ELR-containing CXC chemokine ligands are critical for the recruitment of neutrophils in many models. Importantly, human and mouse ELR-containing CXC chemokine ligands are not precise structural homologues, complicating the application of experimental animal data to human disease: human ligands (CXCL1/GROα, CXCL2/GROβ, CXCL3/GROγ, CXCL5/ENA-78, CXCL6/GCP-2, CXCL7/NAP-2, and CXCL8/IL-8) can signal via two receptors, CXCR1 and CXCR2. Mouse ligands (CXCL1/KC and CXCL2/MIP-2, CXCL5/LIX, CXCL6/GCP-2, and CXCL15/lungkine) all signal via a single receptor, CXCR2 (19). In wild-type mice challenged with large intratracheal inocula of *Aspergillus* conidia, there is a marked induction of the ELR-containing CXC chemokine ligands CXCL1/KC and CXCL2/MIP-2 that was associated with a rapid recruitment of neutrophils to the lungs (104). In these animals, the immunoneutralization of CXCR2 resulted in a marked impairment of neutrophil influx to the site of infection in immunocompetent animals, resulting in severe invasive aspergillosis with nearly 100% mortality (104); similarly, CXCR2-deficient animals challenged with intrapulmonary conidia had an impaired recruitment of neutrophils to the lungs associated with conidial germination in the lungs (19). Conversely, the transient overexpression of CXCL1/KC in the lungs of immunocompromised mice resulted in lower mortality rates and lower lung fungal contents, even when the transgenic expression of the ligand began after the infection had been established (106). Unexpectedly, the transgenic expression of CXCL1/KC also resulted in a greater accumulation of monocytes/macrophages at the site of infection that was associated with a greater local expression of IFN-γ and IL-12p70, suggesting that the increased numbers of lung neutrophils in transgenic animals exerted a beneficial immunomodulatory effect in addition to direct neutrophil-mediated fungal killing.

Recruitment of Mononuclear Cells

The CXCR3 chemokine ligands CXCL9/MIG, CXCL10/IP-10, and CXCL11/I-TAC represent a unique group of ELR-negative CXC chemokines that are potently induced by both type I and type II IFNs. Although the role of these chemokines in the host defense against *Aspergillus* has not been studied in detail, they are induced in neutropenic mice with invasive aspergillosis (121). In addition, a single-nucleotide polymorphism associated with reduced levels of expression of CXCL10/IP-10 has been associated with an increased risk of invasive aspergillosis in allogeneic stem cell transplant recipients, providing a potential relevance of these chemokines to human infection (109).

The CC chemokine ligand CCL3/MIP-1α signals via two receptors, CCR1 and CCR5. In mice with antibody-mediated neutrophil depletion and invasive aspergillosis, CCL3/MIP-1α was markedly induced in the lungs, and its neutralization resulted in increased lung fungal content and increased mortality that was associated with reduced numbers of lung monocytes/macrophages (103). Interestingly, immunocompetent CCR1-deficient mice inoculated with intravenous *Aspergillus* conidia were reported to have an increased susceptibility to the infection that was associated with an impaired trafficking and proliferation of myeloid cells (61), suggesting that a similar mechanism may be operational in the lungs of neutrophil-depleted mice.

CCR6 is a chemokine receptor for the ligand CCL20/MIP-3α as well as several members of the β-defensin family and is expressed on immature dendritic cells, mature B cells, and memory T cells. In the context of mice with antibody-mediated neutrophil depletion and invasive aspergillosis, myeloid dendritic cells were the major population of CCR6-expressing cells in the lungs, and their accumulation in the lungs corresponded to the local induction of the ligand CCL20. Neutropenic mice deficient in CCR6 had worsened survival and greater lung fungal burden associated with fewer lung dendritic cells and recruited monocytes/macrophages; similarly, the neutralization of CCL20 resulted in impaired lung fungal clearance and impaired recruitment of dendritic cells to the lungs during the early phase of infection (125).

CCL2/MCP-1 is another CC chemokine ligand that is markedly induced in the lungs of both immunocompetent mice and mice with antibody-mediated neutrophil depletion challenged with intratracheal *A. fumigatus* conidia (17, 114). The neutralization of CCL2/MCP-1 in neutrophil-depleted mice resulted in an increased severity of infection that was associated with a markedly reduced accumulation of classical NK cells in the lungs but surprisingly did not affect other leukocyte subsets. The transfer of labeled NK cells to infected mice resulted in their accumulation in the lungs, but this effect was attenuated with CCL2/MCP-1 neutralization, indicating that this ligand is necessary for the influx of these cells to the lungs. Moreover, the expression of CCR2, the only known receptor for CCL2/MCP-1, on NK cells was also shown to be necessary for the lung influx of NK cells in invasive pulmonary aspergillosis (114). This effect appeared not to represent a direct single-ligand–single-receptor interaction, however, since the neutralization of CCL2 in CCR2-deficient mice resulted in further decreases in lung NK cell influx compared to the absence of CCL2 or CCR2 alone (114).

CCR4 is a CC chemokine receptor that binds several ligands including CCL2/MCP-1, CCL17/TARC, and CCL22/MDC. All of these ligands are induced in the lungs of neutrophil-depleted mice with invasive aspergillosis (30). Surprisingly, the neutralization of CCL17 or deficiency of CCR4 resulted in greater protection from invasive aspergillosis. The precise cellular mechanism of this effect is not yet clear but appears to involve a local immunosuppressive effect, since CCR4-deficient mice had higher lung IL-12 and CCL2 levels that were associated with greater numbers of macrophages and dendritic cells in the lungs (30). Interestingly, despite higher lung CCL2 levels, CCR4-deficient mice had fewer lung NK cells, suggesting that intact CCR4 signaling may be required for optimal NK cell recruitment.

INNATE EFFECTOR MECHANISMS

Alveolar Macrophages

Alveolar macrophages are the major resident leukocytes in the lung and provide an early line of defense against inhaled conidia that have reached the alveoli (149). With a variety of soluble and surface pathogen recognition receptors at their disposal, alveolar macrophages can quickly adhere to and ingest conidia entering the alveolar space (1). Phagocytosis and the secretion of proinflammatory cytokines by alveolar macrophages help to eliminate conidia and restrict the initial spread of microorganisms in the alveoli. Alveolar macrophages are sufficient to overcome small inocula of *Aspergillus* conidia, as demonstrated in a murine model of invasive aspergillosis (40). However, larger challenges of *Aspergillus* conidia evidently overwhelm the capacities of local defenses, necessitating the recruitment of other effector leukocytes. This may be due to the relatively slow killing of conidia by alveolar macrophages: in in vitro studies, conidial killing by alveolar macrophages was delayed 3 to 6 h after phagocytosis (148), corresponding to the time when conidia become swollen.

The biochemical and molecular mechanisms for the killing of phagocytosed conidia have been studied most thoroughly in the context of the study of chronic granulomatous disease. Chronic granulomatous disease results from inherited mutations in any of the four components of the NADP (NADPH) oxidase complex and results in an impaired ability to generate reactive oxygen species and a consequent susceptibility to several infections, most notably invasive aspergillosis (152). Most available evidence suggests that alveolar macrophages can kill conidia via nonoxidative mechanisms: rabbit alveolar macrophages were able to kill *Aspergillus* conidia under anaerobic conditions (148), and human blood monocytes cultured for 10 days (which have a reduced capacity to generate reactive oxygen intermediates [116]) were also able to kill fungal conidia as effectively as human blood monocytes after 2 days of in vitro culture (which have intact reactive oxygen intermediate production [148]). Macrophage colony-stimulating factor-induced *Aspergillus* hyphal damage was observed in conjunction with enhanced superoxide anion production in both human monocyte-derived macrophages and rabbit alveolar macrophages (140). In addition, alveolar macrophages from mice deficient in $gp91^{phox}$ (analogous to human X-linked chronic granulomatous disease) inhibited conidium germination as efficiently as wild-type alveolar macrophages (19, 45, 113). In contrast, alveolar macrophages from mice lacking $p47^{phox}$, another component of the NADPH oxidase complex and mimicking an autosomal recessive form of human chronic granulomatous disease, have been reported to phagocytose conidia normally but are unable to kill them (126). The explanation for this discrepancy may relate to methodological issues or differences in macrophage function in different forms of chronic granulomatous disease.

Several in vitro studies suggested that reactive nitrogen intermediates may also not be necessary for antifungal defense in alveolar macrophages: although IFN-γ-treated alveolar macrophages were shown to have higher levels of nitric oxide production, which was associated with higher rates of killing of *Aspergillus* conidia (66), murine alveolar macrophages did not produce nitric oxide in response to *Aspergillus* conidia (161), and the presence of a competitive inhibitor of nitric oxide synthase did not inhibit conidial killing by human or murine alveolar macrophages (110). In addition, alveolar macrophages from mice deficient in the inducible form of nitric oxide synthase killed conidia as effectively as wild-type alveolar macrophages (126).

Corticosteroid treatment was shown to significantly impair the capacity of killing of conidia by alveolar macrophages. While there was not a significant difference observed with regard to internalizing conidia, alveolar macrophages from corticosteroid-treated mice had more growing fungus, and this

was associated with reduced reactive oxygen species production (126). The altered production of reactive oxygen intermediates may be one contributing factor for the increased susceptibility and development of invasive aspergillosis in corticosteroid-treated mice.

Neutrophils

The duration and extent of neutropenia as well as qualitative defects in neutrophil function are the best-described risk factors for invasive aspergillosis. Defects in neutrophil number and function have long been recognized as being the most pervasive risk factors for the development of invasive aspergillosis in diverse populations of patients including bone marrow recipients (167), patients receiving cytotoxic chemotherapy (63), and patients with chronic granulomatous disease (44) or human immunodeficiency virus (48, 138). Recruited neutrophils were initially thought to act exclusively on hyphae while resident alveolar macrophages killed resting and swollen conidia (149). While neutrophils remain responsible primarily for hyphal killing, they have been shown to have an essential role in killing germinating conidia (19, 84, 92, 175). In contrast to the delayed killing mediated by macrophages, fungal damage and killing by neutrophils are immediate and very rapid (49, 141). Neutrophils bind and internalize swollen conidia to trigger respiratory burst and degranulation (91, 92). The size of the hyphae prevents phagocytosis by neutrophils, but contact between neutrophils and hyphae can induce both oxidative and nonoxidative mechanisms to mediate hyphal damage (49, 141).

The importance of an oxidative killing mechanism in neutrophils is thought to be a major contributor to the susceptibility of patients with chronic granulomatous disease to invasive aspergillosis. Otherwise normal gp91^{phox}-deficient mice are highly susceptible to invasive aspergillosis (5, 19, 113). Since gp91^{phox}-deficient alveolar macrophages can efficiently inhibit growth of *Aspergillus* conidia as well as wild-type alveolar macrophages but neutrophils from gp91^{phox}-deficient mice or chronic granulomatous disease patients have impaired fungicidal activity in vitro, the generation of reactive oxygen species by neutrophils is considered to be the major antifungal mechanism lacking in chronic granulomatous disease. Another major neutrophil effector mechanism is myeloperoxidase, an enzyme stored in neutrophil azurophilic granules that, when released in the context of the oxidative burst, catalyzes the reaction of hydrogen peroxide and chloride anion to generate hydrochlorous acid (82). Otherwise immunocompetent myeloperoxidase-deficient mice are also susceptible to invasive aspergillosis but less so than gp91^{phox}-deficient animals (5). In vitro studies show that neutrophils from mice with a gp91^{phox} deficiency as well as humans with chronic granulomatous disease or myeloperoxidase deficiency are unable to kill hyphae (2, 19, 49, 92). The defect in oxidative killing of *Aspergillus* species by neutrophils from patients with chronic granulomatous disease or myeloperoxidase deficiency can be corrected when as few as 1 normal neutrophil is added to 15 neutrophils from mutant hosts (133), demonstrating cooperation between neutrophils in oxidative killing. In this context, the in vivo neutrophil oxidative burst in response to *Aspergillus* infection appears to occur in the context of intra-alveolar neutrophil aggregates (19). Granulocyte colony-stimulating factor and IFN-γ enhance the neutrophil oxidative response and their ability to kill hyphae, including neutrophils from patients treated with corticosteroids (138, 141, 142). IFN-γ also improves the oxidative response of neutrophils from patients with chronic granulomatous disease, and the prophylactic administration of IFN-γ to patients with chronic granulomatous disease results in a reduction in serious infections, including invasive aspergillosis (56, 76, 151); consistent with this, neutrophils from patients with chronic granulomatous disease who were treated with IFN-γ demonstrated an enhanced ability to kill *Aspergillus* hyphae ex vivo (132).

There is also in vitro evidence to suggest that neutrophils can mediate antifungal activities via nonoxidative mechanisms. Purified neutrophil defensins, which are stored in the primary granule, also have fungicidal activities (93). In addition, neutrophils release lactoferrin from their secondary granules as part of their degranulation when interacting with *Aspergillus* conidia (91, 92). A recent study identified neutrophil lactoferrin sequestration of iron as an important contributor to inhibiting *Aspergillus* conidial growth (175). Cell-free supernatants of degranulated neutrophils from both healthy donors and chronic granulomatous disease patients, which had abundant lactoferrin, were capable of suppressing conidium growth, whereas the presence of ferritin, a soluble iron source, abolished the growth-inhibitory effect on conidia (175). Pentraxins, which have been shown to be critical for mediating resistance to invasive aspergillosis, as discussed above, are also stored in secondary granules and localize to the neutrophil extracellular traps upon neutrophil activation (77).

Recruited Monocytes/Macrophages

Peripheral blood monocytes are a heterogeneous population of myeloid cells that contain the precursors of tissue macrophage and dendritic cells in inflamed tissues (7). Upon interaction with *Aspergillus* conidia, human peripheral blood monocytes undergo profound changes in their expression of hundreds of genes (46, 137). Human monocytes are capable of ingesting and killing conidia and of inducing damage to *Aspergillus* hyphae (50, 140, 156), and this killing can be enhanced in the presence of granulocyte-macrophage colony-stimulating factor, IFN-γ, and fungicidal drugs (41, 52, 139, 166). In the context of neutropenic mice with invasive aspergillosis, these inflammatory mononuclear cells appear in the lungs within hours of the onset of infection (125), but their in vivo role in the defense against invasive aspergillosis has not been directly examined to date.

Natural Killer Cells

NK cells are lymphocytes that were first described as a subset of mouse splenic lymphocytes with spontaneous cytotoxicity against virally infected cells (80, 81). Unlike classical T and B cells, NK cells do not require clonal proliferation before they can respond to antigens; as a result, they can be deployed rapidly as part of the innate effector response. Most of the literature on NK cells has concentrated on cells obtained from the mouse spleen and human peripheral blood, where the cells are easily accessible in relatively large numbers, but NK cells have a broad tissue distribution (65, 134), and after the spleen,

the lungs contain the largest number of tissue NK cells in experimental animals (11, 65, 134, 158). In the uninflamed lung, the majority of NK cells is located in the vascular and interstitial compartments of the lung and is therefore in close proximity to any inhaled microorganisms (11, 171).

In mice with antibody-mediated neutrophil depletion and invasive pulmonary aspergillosis, the additional depletion of NK cells results in worsened outcomes of infection, and accumulation in the lungs is dependent on the chemokine ligand CCL2 (114). In this model, NK cells contributed to early IFN-γ production in the lungs, and the depletion of NK cells or absence of IFN-γ resulted in a similar increase in susceptibility to the infection, whereas the depletion of NK cells in IFN-γ-deficient hosts did not result in a further increase in the severity of infection. Finally, the transfer of activated NK cells from wild-type, but not IFN-γ-deficient, donors resulted in greater pathogen clearance from the lungs of both IFN-γ-deficient and wild-type hosts (121).

Platelets

Several groups have documented the in vitro antimicrobial activity of human platelets against *Aspergillus* species. Platelets bind plasma-opsonized hyphae and degranulate (42). The interaction of platelets with hyphae results in reduced hyphal galactomannan release, impaired hyphal elongation, and loss of hyphal wall integrity, and these effects were inhibited when granule exocytosis was blocked (42, 123). Serotonin, a component of platelet granules, has also been found to kill both conidia and hyphae and to induce damage to fungal cell membranes (88, 124).

Epithelial Cells

The involvement of airway and alveolar epithelial cells in the recognition of *Aspergillus* species has received less attention, although these cells are clearly the first cells to encounter the inhaled organism. In both immunocompetent and neutrophil-depleted mice, we have found histological evidence of the ingestion of fungal elements by ciliated airway epithelial cells (Fig. 3). Similarly, human nasal ciliated epithelial cells phagocytose and kill conidia in vitro (20). A human alveolar epithelial cell line (A549) can bind both *Aspergillus* conidia and hyphae, ingest conidia, and generate IL-6 and CXCL8 in response to them (75, 177). Recent studies of a human bronchial epithelial cell line (BEAS-2B) have also demonstrated a time-dependent synthesis of CXCL8 in response to germinated *Aspergillus* elements (swollen conidia, hyphae, or both) but not resting conidia (9). Interestingly, the epithelial release of CXCL8 was dependent on NF-κB activation but was independent of the TLR-MyD88 pathway, indicating redundant pathways for epithelial recognition and responses to *Aspergillus* species (9).

Cytokines in Innate Leukocyte Activation

The activation of both the resident and recruited leukocytes is, to a large extent, mediated by a diverse set of cytokines that act via autocrine and paracrine effects. The importance of these molecules in the defense against *Aspergillus* species was first noted when the susceptibility of inbred mouse strains was found to correlate with patterns of cytokine production: the production of Th1 cytokines IFN-γ and IL-12p70 correlating with improved outcomes and the production of IL-4 (a Th2 cytokine) associated with a more severe infection (37, 38). As detailed later in this review, the precise role of the Th17 cytokines IL-23 and IL-17 in the host response to *Aspergillus* species is not yet clearly defined. Despite the "Th" designation of these cytokines, note that the host defense mechanisms under discussion here occur within the first days after *Aspergillus* challenge and represent cytokines released from leukocytes in the context of innate immunity.

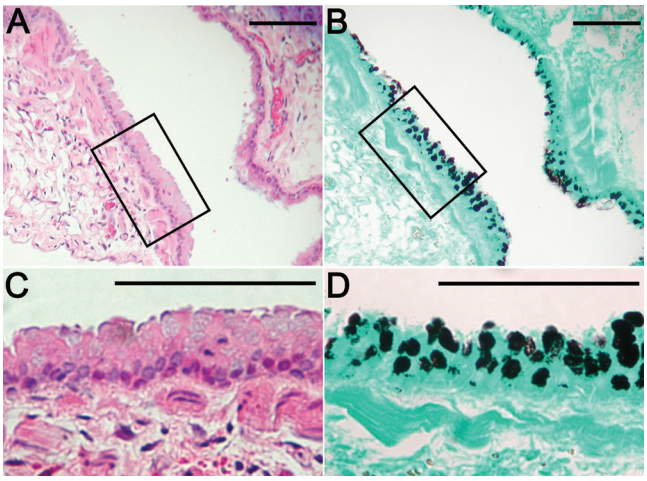

FIG. 3. Association of *Aspergillus* species with bronchial epithelial cells in vivo. Shown is representative lung histology from neutropenic mice 3 days after intratracheal challenge with a sublethal inoculum of *Aspergillus* conidia, with sequential sections stained with hematoxylin and eosin to show mammalian cell morphology (A and C) and Grocott's methenamine silver to demonstrate fungal elements (B and D). (A and B) Extensive association of fungal elements with airway lining. (C and D) Higher-power images of the boxed areas in A and B showing that most of the fungal material appears to be intracellular. All scale bars are 20 μm; original magnifications, ×100 (A and B) and ×400 (C and D).

In the context of early infection, IFN-γ, IL-12, and IL-18 are induced in the lungs of immunocompetent animals in the first 48 h after microbial challenge (26, 37). The administration of neutralizing cytokine-specific antibodies to IL-12p70 or IFN-γ, however, did not influence the clearance of *Aspergillus* from the lungs of immunocompetent mice (26). In contrast, IFN-γ or IL-12p40 knockout animals (the latter deficient in both IL-12p70 and IL-23) that are treated with cyclophosphamide or neutrophil-depleting antibody are more susceptible to invasive aspergillosis (36, 121). Consistent with this, the exogenous administration of IFN-γ results in improved outcomes of experimental infection for mice treated with corticosteroids or cyclophosphamide (115, 155). The cellular source of IFN-γ in late infection and in immunized mice is recognized as *Aspergillus*-specific clones of CD4 T cells in both immunocompetent and cyclophosphamide-treated mice (38, 135, 136), whereas during early infection of neutrophil-depleted mice, the main cellular source of early IFN-γ is lung NK cells (121). The

cellular source of IL-12p70 during in vivo infection is not established, although cultured human and mouse dendritic cells produce this cytokine when exposed to *Aspergillus* conidia in vitro (22, 59, 154), and the transfer of cultured dendritic cells with transgenic expression of IL-12p35 and IL-12p40 to immunocompromised mice with invasive aspergillosis results in an attenuated infection (155).

With regard to Th2 phenotype cytokines, IL-4-deficient cyclophosphamide-treated mice with invasive aspergillosis have reduced mortality and lower lung fungal contents on day 1 of infection than wild-type mice, which are associated with increased lung levels of IL-12p70 and IFN-γ on day 1 of infection. In contrast, IL-5 deficiency had no effect on lung fungal content or survival (36). Additionally, cyclophosphamide-treated IL-4 knockout mice became more susceptible to infection when they were treated with a neutralizing antibody against IL-12p70 (36). These results indicate that in the first day of infection in immunocompromised mice, IL-4, but not IL-5, downregulates the Th1 phenotype in wild-type mice and contributes to the increased severity of infection. The cellular source of IL-4 during in vivo infection has again not been determined, but cultured dendritic cells express IL-4 in response to *Aspergillus* hyphae or proteases (22, 87).

Similar to IL-4, cyclophosphamide-treated IL-10-deficient mice with invasive pulmonary aspergillosis showed enhanced antifungal inflammatory responses, Th1 cytokine production, and reduced severity of infection (47). Immunocompetent IL-10-deficient animals were similarly resistant to infection when given intravenous conidia (43). Consistent with this, the presence of a human IL-10 promoter polymorphism resulting in reduced IL-10 expression levels in recipients of allogeneic bone marrow transplantation was associated with a reduced incidence of invasive aspergillosis (147, 153). IL-6 deficiency in both immunocompetent mice and animals treated with corticosteroids resulted in greater lung fungal contents and reduced survival associated with the impaired conidiocidal activity of lung phagocytes, which was restored after the addition of recombinant IL-6 (35). The cellular sources of IL-10 and IL-6 in invasive aspergillosis remain to be established.

IL-23 is a heterodimer of IL-12p40 and a p19 protein and is important in promoting and maintaining the Th17 phenotype. IL-23-deficient ($p19^{-/-}$) mice or animals with an antibody-mediated blockade of IL-23 or IL-17 in the setting of infection with *Aspergillus* species were reported to have a reduced lung fungal burden; similarly, the neutralization of IL-17 resulted in a greater clearance of *A. fumigatus* from the lungs in $gp46^{phox}$-deficient animals (144, 176). Recent work by another group, however, indicated that the early neutralization of IL-17 resulted in lower lung fungal contents (as determined by fungal RNA) in the first 48 h after intrapulmonary challenge with *A. fumigatus* (172). The reason for these potentially discrepant reports may relate to experimental conditions, including differences in the genetic backgrounds of the mouse strains examined (C57BL/6 versus 129/SvEv), the timing of measurements of lung fungal content (day 3 versus days 1 to 2), or, conceivably, the methods employed to quantify tissue fungal content (lung chitin content versus lung fungal RNA content by quantitative PCR) in the two studies (172, 176).

ROLE OF INNATE IMMUNITY IN SHAPING T-CELL-MEDIATED IMMUNITY

Several lines of evidence support the concept that events during the early innate response to *Aspergillus* species influence the development of subsequent T-cell responses. Pulmonary challenge with *Aspergillus* conidia in immunocompetent mice leads to rapid $CD4^+$ T-cell recruitment into the draining mediastinal lymph nodes, detected as early as 3 days postchallenge and peaking by 7 days after infection (136). $CD4^+$ T-cell recruitment and proliferation in the mediastinal lymph nodes and trafficking to the airways can occur in the absence of MyD88 signaling (135). However, MyD88-mediated signaling is required for the enhanced Th1 differentiation of responding $CD4^+$ T cells in the lymph nodes (135).

Dendritic Cells and Initiation of Acquired Immunity

Dendritic cells have been shown to initiate adaptive immune responses to *Aspergillus* species and to shape the T-cell response to the organism. In vivo and in vitro studies have demonstrated that dendritic cells internalize both *Aspergillus* conidia and hyphae and transport them from the airways to draining lymph nodes (21). The internalization of conidia and hyphae by dendritic cells involves distinct phagocytic mechanisms and pathogen recognition receptors, and this translates into qualitatively different $CD4^+$ T-helper-cell responses: dendritic cells ingest conidia through coiling phagocytosis, in which the extension of unilateral pseudopods rotates around the pathogen to form self-apposed pseudopod layers and involves the ligation of the mannose receptor DC-SIGN and complement receptor 3, leading to the priming of Th1 responses in the draining lymph node and spleen in mice (21). The internalization of hyphae, on the other hand, occurs via the Fc receptor and complement receptor 3-mediated phagocytosis, resulting in a "zipper phagocytosis" that requires attachment through receptor-ligand binding, and engulfment follows the contour of the microorganisms. In contrast to the ingestion of conidia, hyphal phagocytosis by lung dendritic cells results in the production of IL-4 and IL-10 in vitro and the generation of IL-4-producing CD4 T cells in the spleen and mediastinal lymph nodes in vivo (21).

Since dendritic cells have the ability to direct the subsequent development of protective as well as pathological T-cell responses, dendritic cell-based vaccine strategies have been used in proof-of-principle experiments with animal models of invasive aspergillosis: adoptive transfers of dendritic cells pulsed with *Aspergillus* conidia into mice subsequently challenged intravenously with *A. fumigatus* led to improved survival associated with Th1 immunity, with higher IFN-γ production than mice receiving hypha-pulsed dendritic cells (22). The transfer of pulsed dendritic cells also resulted in protection after intrapulmonary infection of mice that were recipients of allogeneic bone marrow transplantation; interestingly, the resistance to infection in this setting exceeded the effect of the adoptive transfer of *Aspergillus*-specific Th1 cells (22, 34). Thymosin-α1, a peptide that augments a number of aspects of T-cell-mediated immunity via undefined mechanisms, also promotes Th1 immunity by enhancing dendritic cell IL-12p70 production in

response to *Aspergillus* and promotes the development of IFN-γ-producing T cells in mice with bone marrow transplantation with invasive aspergillosis (143).

Innate immune mechanisms may also regulate the development of T-regulatory cells against *Aspergillus* species. In immunocompetent wild-type mice, an early population of lung $CD4^+$ $CD25^+$ cells inhibits local inflammation, including TNF production and the generation of reactive oxygen intermediates, via the induction of IL-10 expression and CTLA-4 that was dependent on the costimulatory molecule CD80 (111). The depletion of this population with cyclophosphamide or anti-CD25 resulted in greater lung inflammation. In the allergic model, a second population of regulatory T cells could inhibit the Th2 response, and its development is dependent on the effect of early lung IFN-γ on dendritic cells (111).

As noted above, the role of Th17 cytokines in the defense against *Aspergillus* species remains to be established. The development of Th17-polarized immunity in response to *Aspergillus*, however, appears to be related to events during early innate immunity. Human monocyte-derived dendritic cells have been shown to generate both IL-12p70 and IL-23 when incubated with *Aspergillus* conidia (59). Mice lacking IL-23p19 or with an antibody-mediated blockade of IL-23 or IL-17 in the setting of infection with *Aspergillus* species displayed higher levels of lung IL-12p70 production and IFN-γ-producing $CD4^+$ T cells (176). During heightened fungal growth, dendritic cells were shown to produce IL-23, acting possibly as a positive-feedback loop for further IL-23 production, and neutrophil fungicidal activity, at least in vitro, was impaired in the presence of IL-23 and IL-17 (176). These observations suggest that the development of Th17 immunity inhibits the development of protective Th1 immunity. However, the blockade of IL-23 via neutralizing antibody in IFN-γ-deficient mice with invasive aspergillosis resulted in a further increased fungal burden (176), suggesting that in the absence of effective Th1 immunity at least, the IL-23/IL-17 pathway is protective against *Aspergillus* infection.

Neutrophils in Acquired Immunity

Evidence from in vitro studies suggests that functionally active *Aspergillus*-specific T cells can contribute to enhanced neutrophil effector functions against *Aspergillus*: the combination of human anti-*Aspergillus* T cells and monocyte-derived antigen-presenting cells with neutrophils resulted in significantly increased hyphal damage compared with T cells coincubated with antigen-presenting cells, with neutrophils alone, or with either T cells or antigen-presenting cells (12). This coordinated action between neutrophils and protective Th1 cells for full protection against *Aspergillus* species was also observed in vivo in mouse bone marrow transplant recipients with invasive aspergillosis (143). Conversely, neutrophils exposed to IL-23 or IL-17 were reported to induce less effective fungal killing (176).

Finally, in the context of a murine model of allergic hypersensitivity to *Aspergillus* species, the presence of neutrophils was found to contribute to disease pathology (122): the depletion of neutrophils in this model resulted in reduced airway fibrosis and hyperresponsiveness, whereas an enhanced recruitment of neutrophils to the airway walls by the transgenic overexpression of CXCL1/KC led to an exacerbation of methacholine-induced airway hyperreactivity and greater airway remodeling. These effects appeared to be independent of Th1 and Th2 cytokines but appeared to be dependent on neutrophil matrix metalloproteinase 9 production (122).

CONCLUSIONS

Healthy hosts have evolved multiple layers of innate immune responses that readily clear inhaled *Aspergillus* conidia without developing disease and without the development of acquired immunity to this organism. While many aspects of the host's response to this microorganism remain to be defined, the current data support the hypothesis that the failure of the host's innate defense mechanisms together with the microorganism's ability to adapt to surviving in harsh environments result in the strikingly diverse set of human diseases caused by *Aspergillus* species.

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