Infection or hypersensitivity: Lessons from *Aspergillus* lung diseases

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Introduction

Aspergillus species are ubiquitous environmental molds that are responsible for a remarkably diverse human diseases. I will discuss these diseases in 3 categories:

1. Colonization of mucosal surfaces without invasion into tissue
2. Invasive infection: growth of fungal elements in tissue
3. Hypersensitivity: diseases caused by the immune response of the host

The historical criteria of establishing microbial pathogenesis were developed by Henle in 1840 and made famous by Robert Koch while proving the bacterial pathogenesis of anthrax in 1876 (Table 1). The view of infectious diseases during this period focused on characteristics of the pathogen that result in disease; these features were cumulatively thought of as the microbe's virulence. Examples of such virulence factors include the pneumococcal capsule and diphtherial exotoxin: microbial strains that lack these virulence factors do not cause disease, and introduction of the virulence factors into such strains in the laboratory renders them pathogenic. The diversity of diseases caused by *Aspergillus* species, and the fact that these diseases do not fulfill Koch's postulates, underscores a modification to this historical paradigm: that the disease phenotype, and indeed whether a disease occurs at all, depends not only on what the pathogen does to the host, but also on what the host does in response.

1. Organism is present in every case of disease
2. Organism can be isolated from every case of disease in pure culture
3. Disease can be reproduced by inoculating the pure culture into a healthy host
4. Organism can again be recovered from the experimentally infected host

Table 1. Koch's postulates for establishing a causal relationship between a pathogen and a disease

In this grand rounds, I propose to explore how the host's response to *Aspergillus* might explain these varied disease phenotypes. I will begin by introducing the pathogen and the generic host response to pathogens in the lung. I will then review the salient clinical features of the different diseases caused by *Aspergillus* species, and discuss how aberrations in the host response might account for them.

A. The pathogen:

Fungi are a large and diverse kingdom of eukaryotic organisms, characterized by presence of a cell wall, lack of chlorophyll or flagellae, and reproduction by means of spores. Only a tiny subset of fungi cause human disease. A useful classification of these medically relevant species is on the basis of morphology, as species that are always yeasts (e.g. *Cryptococcus neoformans*), those that are always molds (e.g. *Aspergillus* species), and dimorphic organisms that have mold morphology in nature but assume yeast morphology when they infect a host (e .g. *Histoplasma capsulatum*). Yeasts are rounded or elongated single cells that typically reproduce by budding, and form colonies with macroscopically smooth surfaces. Molds grow as branching multi-cellular filamentous structures termed hyphae. Large numbers of hyphae growing together are referred to as a mycelium, and have a hairy or fuzzy appearance macroscopically (Figure 1). In the case of *Aspergillus* species, specialized hyphae give rise to reproductive structures, called conidiophores, which in turn produce the reproductive spores, the conidia. The microscopic appearance of these reproductive structures is one of the main criteria used for identification of various mold species.

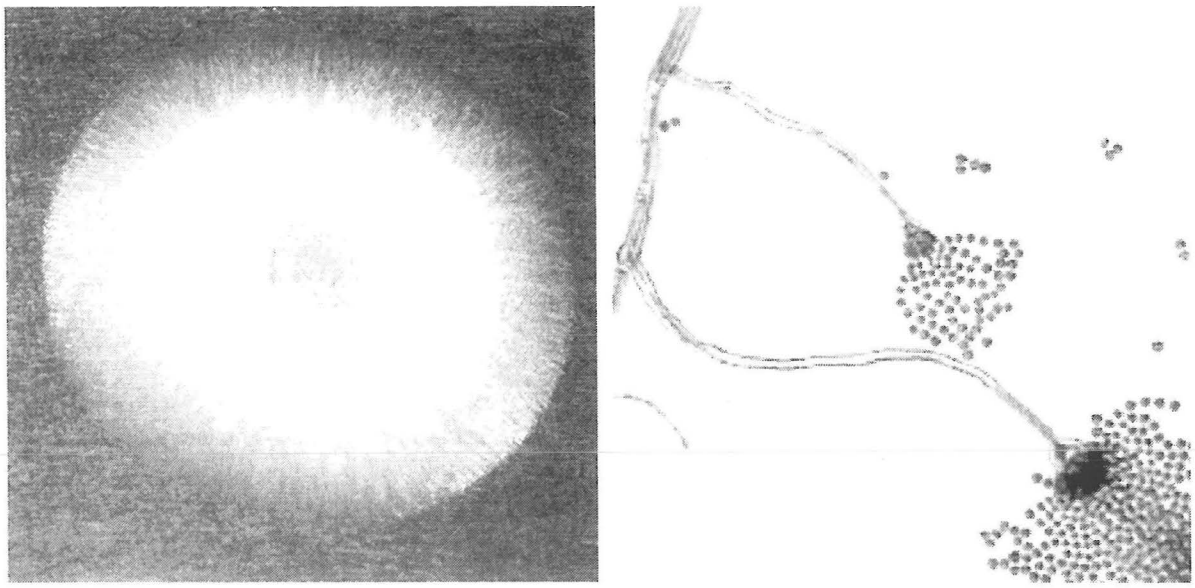

Figure 1. Macroscopic and microscopic appearance of *A. fumigatus* in culture

The conidia of *Aspergillus* species are 2-5μm in diameter and have a rough and hydrophobic exterior, characteristics that make them easily airborne. They are released in large numbers from the mycelium in bursts caused by minor air currents and, once disturbed, remain in the air for hours. Spores that settle but do not germinate remain viable for months, and become airborne again easily. As a result, the conidia are extensively disseminated in the environment and are, for practical purposes, unavoidable (1).

The usual habitat of *Aspergillus* species is decaying organic material in the soil, but their minimal culture requirements and efficient dissemination allows growth in all manner of conditions, and many studies have documented their isolation from alarming or unlikely sources: the organism has been isolated from hospital construction materials and ventilation ducts, ornamental plants and numerous cooking ingredients, marijuana, Antarctic snow, and the interior of orbiting spacecrafts (2-12). Due to the episodic nature of the release of conidia into the environment, systematic studies of air samples for the organism are often limited by sampling errors . With this caveat in mind, mean outdoor concentration of *Aspergillus* conidia has been estimated as $0.2\text{-}25/m^3$ in different studies (13-15), and as many as $10^6/m^3$ in some agricultural environments (16). *Aspergillus* species are also among the most prevalent indoor molds (17). It is estimated that we all inhale a few hundred conidia every day.

Not surprisingly, most human diseases caused by *Aspergillus* species involve the respiratory tract or the paranasal sinuses. With the exception of hematogenous dissemination from the lung, primary infections of other organs involve direct inoculation of the organism and are rare. Among the *Aspergillus* species, at least 90% of human disease is caused by *A. fumigatus*. *A. flavus* accounts for most of the remaining cases; *A. Terreus* and *A. Niger* infections are very rare. The reasons for the dominance of *A. fumigatus* are not completely clear, but may in part be due to the particularly small size of *A. fumigatus* conidia (2-3μm) allowing easy access to the lower respiratory tract. In addition, unlike most other molds, *A. fumigatus* is particularly thermotolerant and grows well at up to 45°C.

B. The host's response:

The respiratory tract represents the largest interface between the body and the environment, and its defense against environmental pathogens is a formidable task. Consider the scope of the problem: each day, an interface with a surface area of $70m^2$ is exposed to 8000L of air on one side and 7000L of blood on the other, while a barrier with a mean thickness of

0.6μm separates the two. Clearly, pathogens have to be eliminated before they can inhabit or breach the barrier, but this must be achieved without disruption of the gas exchange surface. The importance of this balancing act is underlined by two of the major categories of lung disorders, infections and immunologically-mediated diseases: failure to clear the pathogen will result in pneumonia, too great an inflammatory response to the pathogen will result in acute lung injury and ARDS, and inappropriate response to innocuous inhaled antigens leads to a variety of hypersensitivity lung diseases.

The elements of lung host defense are summarized in Figure 2. The host defense against a pathogen can be divided into innate and acquired immune responses. As the first line of defense, innate immunity provides recognition of a pathogen followed by rapid mobilization of an "effector" arm that is often sufficient to kill the pathogen. Acquired (or adaptive) immunity, activated in parallel with the innate response, relies on clonal expansion of antigen-specific lymphocytes; this process takes several days but provides lasting immunity.

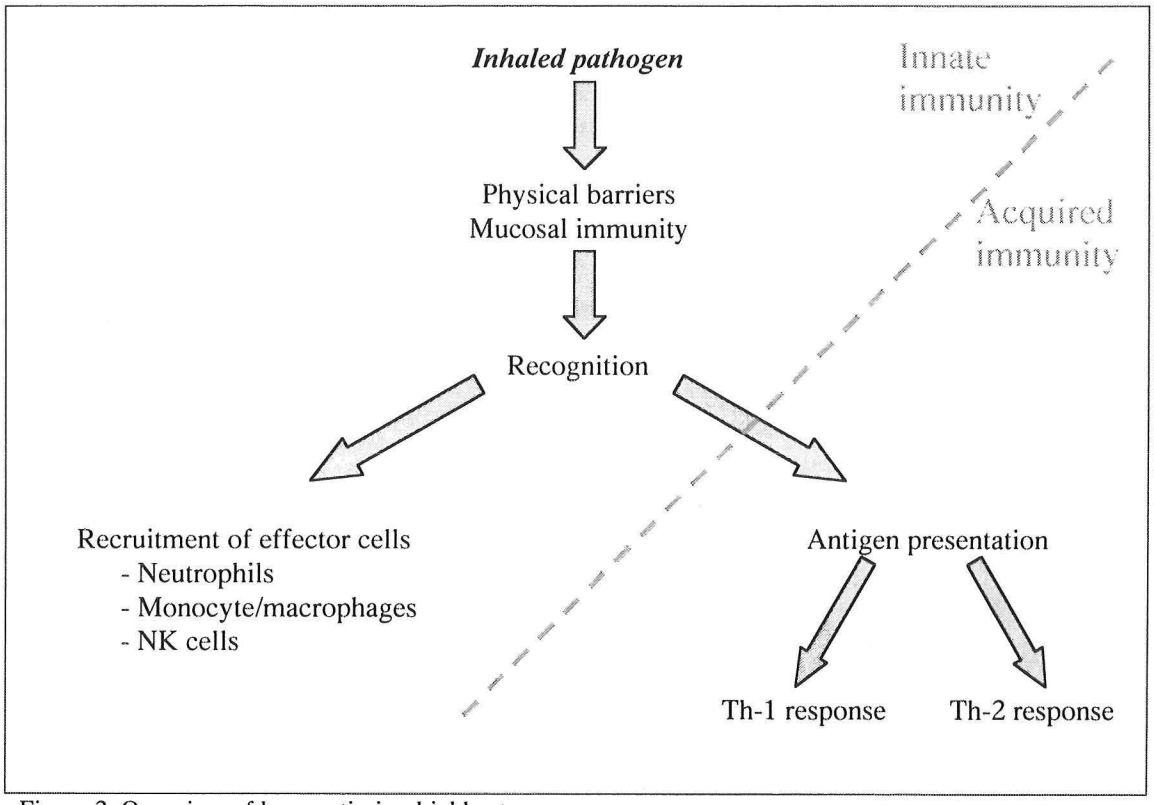

Figure 2. Overview of lung antimicrobial host response

The highly turbulent inspiratory airflow and the repeated branching of the airways result in deposition of particles >5μm in diameter on the mucus barrier that coats the epithelium; these particles are then physically removed by the cough reflex and the ciliary action of the respiratory epithelium. In addition, the surface fluid contains several classes of antimicrobial molecules that help to kill the pathogens. Particles <5μm in diameter, including many respiratory pathogens, bypass the physical barrier of the mucociliary ladder and are deposited beyond the ciliated epithelium in the terminal airways.

At this level, the alveolar macrophages are a critical component of the lung's innate immunity. Organisms that reach this level (including *Aspergillus* conidia) are recognized and rapidly phagocytosed by these cells and most are killed via both oxidative and non-oxidative mechanisms. The recognition of the pathogen by the innate immune system is achieved by means of host molecules that recognize molecular patterns that are invariant components of

entire classes of pathogens – such as LPS for Gram-negative bacteria, or lipoarabinomannans for mycobacteria – but which do not occur in mammalian cells. Some of these "pattern-recognition" molecules are dissolved in tissue fluids; after binding the pathogen, these molecules might promote their phagocytosis or activate the complement cascade. Pattern-recognition molecules can also be receptors on the surface of cells such as alveolar macrophages; recognition of a pathogen by different classes of these receptors triggers various actions, such as phagocytosis, by the cell. One class of these pattern-recognition receptors, the Toll-like receptors (TLRs), initiate the synthesis and release of inflammatory mediators by innate immune cells. Different members of the TLR receptors recognize different classes of pathogens; TLR4, for example, is responsible for the inflammatory response to LPS from Gram-negative organisms – mice deficient in TLR4 do not have the normal brisk inflammatory response to LPS, and are extremely susceptible to Gram-negative infections (18, 19).

Once activated, resident innate immune cells such as alveolar macrophages initiates a complex response by releasing diverse classes of inflammatory mediators, including arachadonic acid metabolites, antimicrobial peptides, and cytokines. These mediators have pleotropic effects which ultimately result in changes in the local environment that limit microbial growth, keep the tissue damage localized, and recruit and activate effector cells such as neutrophils, macrophages, and NK cells to the site of infection.

As part of this innate immune response, pathogens that are being encountered for the first time are processed by antigen-presenting cells such as immature dendritic cells. Upon exposure to a pathogen, these cells mature, move to lymphatic tissue, and induce the activation and proliferation of the few T-cell clones whose receptors recognize the antigens from the pathogen. Naïve CD4 T-cells activated in this way can differentiate into one of two, mutually exclusive, phenotypes: the Th-1 cells activate macrophages, induce B cells to release IgG, and mediate the release of IL-12 and interferon-γ. Th-2 cells induce B-cells to release IgE, mediate the release of IL-4, IL-5, and IL-13, which in turn recruit eosinophils to the site on inflammation. The events that determine whether a naïve CD4 T cell develops into a Th-1 or Th-2 have not been fully worked out but are of great interest. Many infectious diseases are the consequence of an inappropriate Th-2 (instead of a Th-1) phenotype acquired immune response to the pathogen, resulting in failure to clear the infection; leprosy, leischmaniasis, and cryptococcosis are examples of this phenomenon. Conversely, many hypersensitivity diseases are characterized by inappropriate on-going Th-1 or Th-2 immune responses: Multiple sclerosis and hypersensitivity pneumonitis, for example, are characterized by pathological Th-1 responses, whereas ulcerative colitis and asthma are mediated by inappropriate Th-2 immunity.

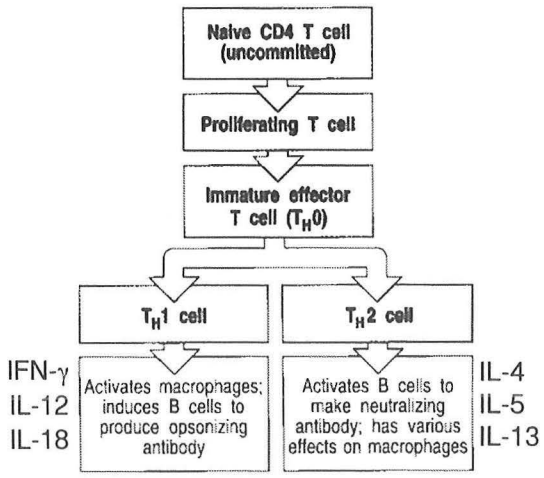

Figure 3. CD4 T cell differentiation. From (20).

Colonization

A. Clinical review:

Asymptomatic colonization of the respiratory tract with *Aspergillus* species is relatively common in the setting of underlying lung disease. Typically, the organism is unexpectedly recovered on bacterial respiratory culture in the occasional patient with bronchogenic carcinoma, fibrocavitary lung disease, COPD, or bronchiectasis. In a study of respiratory sample that grew *Aspergillus* species in a microbiology referral lab, 36% were found to be from such colonized patients (21). *Aspergillus* colonization has been reported to occur at some point in 26% of lung transplant recipients (22) and 30-40% of patients with cystic fibrosis (23, 24). The vast majority of such asymptomatically colonization patients remain well, and they should therefore not be treated.

Colonization of a pre-existing lung cavity with a macroscopically visible ball of mold was first described in 1938 (25) and was the most commonly recognized form of *Aspergillus* lung disease in the 1950's. Since the disease is caused by *Aspergillus* molds in the vast majority circumstances, the terms aspergilloma, fungus ball, and pulmonary mycetoma are essentially interchangeable. The "ball," which consists of mass of live and dead hyphae and proteinacious exudate, can reach several centimeters in diameter. Any poorly-drained space in the lung can be affected; the commonest is still fibrocavitary sequelae of tuberculosis, followed by lung cavities from advanced sarcoidosis, histoplasmosis, *Pneumocystis* infection, or abscesses, dilated airways in bronchiectasis of any cause, and emphysematous bullae (26-30).

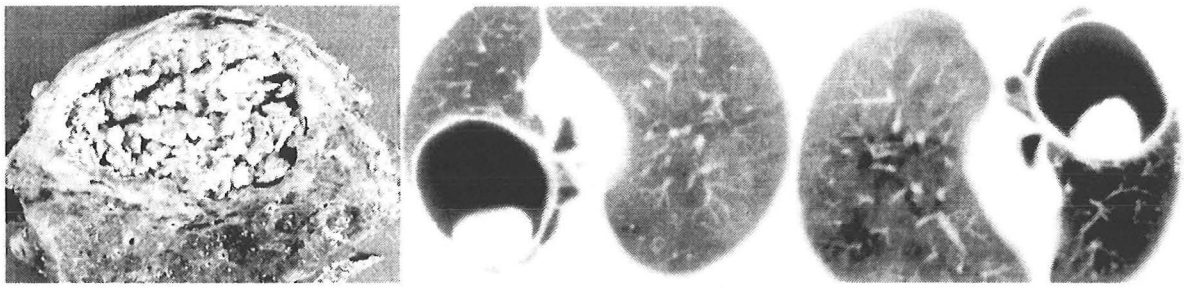

Figure 4. Gross pathology and CT appearance of an aspergilloma. Note that the mycetoma is mobile within the lung cavity.

Most of the clinical data on this disease is from retrospective case-series. The best data on prevalence is from a series of 544 patients with tuberculous cavities >2.5cm in diameter, 11% of whom had radiologic evidence of a mycetoma (31). Most patients have cough, dyspnea, and sputum production, and 10-15% of patients have fever and weight loss. Hemoptysis occurs in 60-95% of patients at some point and usually consists of minor blood-streaking of sputum (26, 27, 29, 30, 32-34). Five to 10% of patients, however, have brisk and large volume hemoptysis; massive hemoptysis is the cause of death in 10-25% of patients with aspergillomas (32, 33). The sources of bleeding are blood vessels in the wall of the cavity which are supplied by tributaries of the bronchial and chest wall circulation, and there have systemic (rather than pulmonary) arterial pressures (35). The diagnosis of an aspergilloma is based on radiographic demonstration of a rounded mass within a cavity; the mass is usually mobile and is occasionally partially calcified. An early clue on the chest radiograph is adjacent thickening of the pleural; asymmetric apical pleural reaction in a patient with bilateral upper lobe fibrocavitary process is a classic presentation. The organism can be cultures from the sputum in about half of the patients, and nearly all have serum antibodies to *Aspergillus* species, detectable either by serum precipitin test or *Aspergillus*-specific IgG analysis (28, 30-33, 36).

The dilemma in this disease is the treatment. Case-reports and small uncontrolled series have reported variable success with systemic or intra-cavitary amphotericin B or itraconazole (36-41). Apart from major methodologic flaws and publication bias in favor of positive results,

these reports are limited by the observation that about 10% of pulmonary mycetomas disappear without therapy (32). Embolization of the bleeding artery can be a life-saving measure in patients with on-going massive hemoptysis, but is complicated by difficulty in finding the bleeding vessel, which may be a tributary of bronchial or chest wall arteries; in addition, hemoptysis often recurs due to development of collateral flow (35). Surgical lung resection of the affected lung is definitive, but is associated with high complication rate due to the underlying lung disease and presence of highly vascular pleural adhesions. In retrospective series, perioperative mortality has been reported as 5-10% and major complications in another 15-30%, with worse outcomes occurring in patients with poorer lung function and complex and thick-walled cavities (29, 34, 42, 43). With this in mind, asymptomatic patients are observed, and patients with severe hemoptysis are referred for surgery if they have good lung function and simple, thin-walled cavities. Management of patients with mild hemoptysis who are good surgical candidates, and those with severe hemoptysis who are poor surgical candidates, is difficult.

B. Colonization and surface immunity:

What defects in host defense might account for *Aspergillus* airway colonization? Nearly all patients with airway colonization have an abnormalities of the respiratory mucosa: histologically, there is varying degrees of epithelial squamous metaplasia in bronchiectasis, chronic bronchitis, and lung cavities. Lung-transplant recipients have a suture-line in the airway. A reasonable hypothesis might be that some feature of the normal respiratory epithelium, absent in these patients, protects against *Aspergillus* colonization.

One such feature is the antimicrobial properties of the small amount of fluid that lines the entire respiratory tract, the so called airway surface fluid (ASF). In addition to physically catching inhaled organisms in a glycoprotein matrix which is then subject to physical removal by ciliary action and cough, the ASF contains a complex mix of many classes of constitutive and inducible antimicrobial molecules, examples of which are given in Table 2.

A major class of ASF antimicrobial peptides are the defensins, a group of small and highly cationic peptides (reviewed in 44). In addition to expression in the respiratory epithelium, members of this family are expressed by gastrointestinal and urogenital epithelia, as well as some leukocyte subsets. Defensins exert direct antimicrobial effects against a broad group of pathogens (including fungi) by permeabilizing biological membranes. In addition, these molecules activate the complement cascade, stimulate the secretion of chemokines such as IL-8 from

Molecule	Antimicrobial action
Cathelicidin	Directly microbiocidal
Collectins (surfactant proteins A and D)	Opsonization chemotaxis modulate cytokine response
Complement	Opsonization chemotaxis membrane permeabilization
Defensins	Opsonization chemotaxis modulate cytokine response
Fibronectin	Opsonization
Immunoglobulins (Ig A in bronchial fluid; Ig G in alveolar fluid)	Opsonization complement activation
Lactoferrin	sequesters iron from use by pathogens
Lysozyme	Digests microbial structures
Nitric oxide	Directly microbiocidal

Table 2. Partial list of antimicrobial products in airway surface fluid

neighboring cells, and up-regulate the expression of adhesion molecules on blood vessel endothelium, thereby promoting the inflammatory response to an inhaled pathogen. An intriguing observation about several classes of surface peptides, including the defensins, is that their net positive charge is critical to their antimicrobial action by directing them to negatively charged moieties on the surface of pathogens. As a result, the effect of these molecules on pathogens is progressively inhibited in increasing ionic concentrations, as the anions and cations in the fluid neutralize the charges of the peptide and the microbial surface. The salt concentration of ASF is normally tightly regulated, but is markedly elevated in cystic fibrosis due to mutations in a chloride channel (CFTR) expressed on respiratory epithelial cells. In support of this hypothesis, cystic fibrosis respiratory epithelium has been shown to express normal amounts of defensins with reduced microbiocidal properties. Cystic fibrosis defensins regain their microbiocidal properties if put in hypotonic solutions, and normal defensins loose their microbiocidal action when placed in hypertonic environments (45, 46).Table 2.

Invasive infection

A. Epidemiology and risk factors:

Invasive aspergillosis (IA) is a relatively common disease with a very poor outcome. Since its first description in 1953 in a patient treated with corticosteroids (47) the incidence of the disease has increased steadily due to the increasing number of immunocompromised hosts: autopsy series at different institutions found a 158% increase between 1960 and 1970, and 132% increase between 1978 and 1992 (48, 49). The mortality data are even more alarming: according to analysis of US death certificates, death from invasive aspergillosis has increased by 357% between 1980 and 1996 (50). In a recent systematic review of published case series, the case-fatality rate was 50-90% depending on the underlying disease; two-thirds of the patients died of the disease despite appropriate therapy (51). In another review of the literature, the crude mortality of pulmonary and CNS involvement were 86% and 99%, respectively (52). In 1996, US hospitalizations for this diagnosis cost $633 million (53).

The most important and widely recognized risk factor for IA are defects in neutrophil number or function – patients at highest risk have an absolute circulating neutrophil count <100 cells/L for >12 days. Other defects in cellular immunity are less readily detectable but also play an important role: for example, recipients of solid-organ transplantation, specially lung transplant patients, are susceptible to invasive infection without neutropenia. As another example, about half of the patient with advanced AIDS who developed IA had normal neutrophil counts (54, 55). Even patients with neutropenia often have co-existing abnormalities in other immune cell lines. Infections in immunocompetent patients are far less common, and are generally the result of direct inoculation of the organism into tissue (e.g. surgical wound infection or line sepsis) or abnormalities in surface defenses (e.g. skin infection in burn patients). A few case reports have described invasive infection in completely healthy individuals (56).

Malignancy
Leukemias and lymphomas
other malignancy (usually related to chemotherapy)
Transplant recipients
Allogeneic bone marrow
Lung or heart-lung
Other organ transplantation
Other immunocompromised hosts
Advanced AIDS (pre-HAART era)
Aplastic anemia
Immunosuppressive therapy
Inherited immunodeficiency states
Patients not immunocompromised by usual criteria
Lung disease (e.g. COPD)
Direct inoculation of pathogen (e.g. IVDA, line infection)

Table 3. Patients at risk for invasive infection

B. Clinical syndromes and diagnosis:

The respiratory tract is the most common site of both primary infection and dissemination in invasive aspergillosis. There is a broad spectrum of acuity of invasive *Aspergillus* pneumonia: at one extreme, patients with severe and prolonged neutropenia can develop an acute pneumonia which is often disseminated on presentation and can result in death within 1-2 weeks of diagnosis. The symptoms are those of any acute pneumonia; fever might be absent or else the disease can present as a neutropenic fever. Infarction of lung segments as a result of invasion of blood vessels can result is hemoptysis that can occasionally be massive and life-threatening (57). At the other extreme of acuity, chronic necrotizing aspergillosis (CNA) is a chronic pneumonia that slowly progresses over many months. CNA affects patients with more intact immune systems: the most common underlying diseases are structural lung diseases (specially COPD and interstitial lung disease) and prolonged systemic therapy with corticosteroids. Notably, CNA can develop in patients with pre-existing pulmonary aspergilloma that begins to invade the surrounding lung parenchyma (58, 59), in addition to patients with connective tissue diseases, diabetes, and alcohol abuse.

Another respiratory syndrome is an invasive endobronchial infection, which can occur in isolation or in association with invasive pneumonia. This syndrome can occur in any of the risk groups, but is over-represented in patients with advanced AIDS (54, 55) and in recipients of lung transplantation, in whom it generally involves the bronchial anastomosis (22). The infection can range in severity from an ulcer or plaque resulting in cough and dyspnoea to an extensive pseudomembranous tracheobronchitis resulting in airway obstruction.

Hematogenous dissemination of IA most commonly involves the lungs and brain, but can spread to virtually any organ. Spread to skin, endocarditis, endophthalmitis, and osteomyleitis are all rare but well-described. Similarly, contiguous involvement of pleura, mediastinum, and chest wall rarely occur.

Respiratory tract
Acute pneumonia
Chronic necrotizing pneumonia
Endobronchial infection
Invasive rhinosinusitis
Hematogenous dissemination
Lung
Brain
Endocarditis, skin, endophthalmitis
Direct inoculation of other organs
Keratitis/ophthalmitis
Skin (wound infection, line sepsis, burns)
Contiguous spread
Pleura, mediastinum, chest wall from pneumonia
Bone, meninges, brain from sinusitis

Table 4. Patterns of invasive aspergillosis

A major obstacle in the management of invasive aspergillosis is delayed diagnosis. The contention that early treatment improves the outcome of the disease is not proven but seems intuitive and is widely accepted. Since the patients are usually immunocompromised and the pace of illness is often rapid, and aggressive approach is necessary. Radiographic appearance of invasive pneumonia are usually not specific, and range from focal air-space disease, single or multiple nodules which may be cavitary, to bilateral extensive airspace disease. Three

radiographic signs on chest CT are, in the appropriate setting, very highly suggestive of this infection (Figure 5). The halo sign refers to a rim of ground glass attenuation surrounding a parenchymal nodule or mass; the sign represents hemorrhagic pulmonary infarction due to angio-invasive disease. Infarction of a peripheral lobule will give the appearance of a wedge shaped pleural-based opacity. Finally, the infarcted lung can shrink and separate from the normal surrounding lung, producing an air crescent. Note that this air crescent is not due to a pulmonary mycetoma discussed earlier – the "ball" in this case consists of infarcted lung, rather than a mass of hyphae growing in a pre-existing lung cavity.

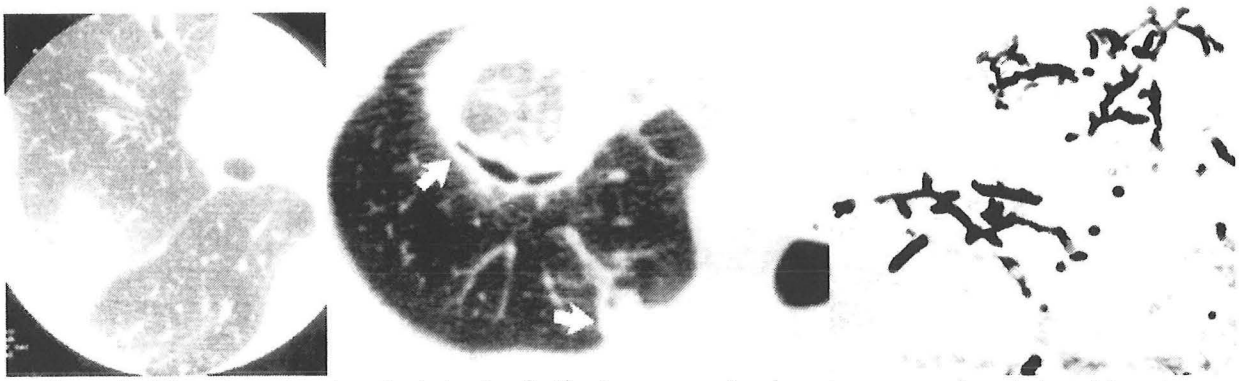

Figure 5. CT sections showing the halo sign (left), air-crescent sign (center, top arrow), and pleural-based infiltrate (center, lower arrow). Histopathologic appearance of pulmonary IA (right) GMS stain, x400.

In the typical patient, several days elapse between the time that IA is considered and the time that the diagnosis is established, during which the patient should be on treatment. Definitive diagnosis requires both histologic evidence of tissue invasion and isolation of the organism in culture. The typical histologic appearance is of non-pigmented, non-septate, dichotamously branching hyphae that are 2-5 μm in diameter, but this appearance cannot be distinguished from other invasive mold infections, such as *Fusarium* and *Pseudallescheria/Scedosporium* species. In patients at high risk, the culture of *Aspergillus* species from respiratory samples even is the absence of histology is highly suggestive of infection, and is certainly sufficient reason to institute therapy. In allogeneic bone marrow transplant recipients, for example, the positive predictive value of positive respiratory cultures for invasive infection is 70% (60). Bronchoscopy is a safe and effective means of obtaining respiratory samples in this setting, with a reported complication rate of 2.5% in immunocompromised patients with pneumonia (61, 62). CT-guided fine needle aspiration is similarly effective, but is complicated by pneumothoraces in at least 10-20% patients (63, 64). Transbronchial biopsies and FNA are contraindicated in severe thrombocytopenia or coagulopathy, but bronchoalveolar lavage can still be performed.

A number of serologic tests for the infection are under development to help with the early diagnosis of IA. Antibody tests have been disappointing because many patients at risk of disease do not develop a normal antibody response. Antigen tests have shown more promise: galactomannan, a component of hyphal wall, is released in large quantities when *Aspergillus* species grow in vitro, and is detectable in small concentrations in the serum of experimentally infected animals (65). A latex agglutination test for galactomannan is very specific (90-100%) but not sufficiently sensitive (30-50%) (66-68). An ELISA for galactomannan, commercially available in Europe, is highly sensitive but has a 10% false-positive rate in hematology patients (69-75). β 1,3-d-glucan, another hyphal wall component, can be detected using a highly sensitive assay similar to the limulus lysate assay used for detection of LPS. In this assay, 1,3-β-glucan in serum activates a cascade of proteolytic enzymes in vitro, the final product of which is detected colorimetrically. While very sensitive, this assay does not differentiate between different fungal species. PCR-based assays that look for fungal DNA or ribosomal RNA segments are also under study.

C. Treatment:

Several major hurdles stand in the way of evaluating treatments for IA. A fundamental issue is that the correlation between the in vitro action of a drug against the organism and its in vivo effectiveness in an infection has not been established. This lack of correlation is the result of lack of standardization of the in vitro tests, pharmacokinetic and pharmacodynamics of the drug, and the effects of host response. Another important problem is the uniformly poor quality of clinical data for various therapeutic modalities in this disease. Remarkably, a recently published IDSA-sponsored practice guideline for treatment of *Aspergillus* diseases (41) did not contain a single recommendation supported by level 1 evidence (evidence from at least one properly controlled randomized trial).

The largest therapeutic experience is with deoxycholate preparations of amphotericin B. Amphotericin B binds to fungal cell membrane sterol molecules, resulting in increased cell permeability and death. In a review of all published series up to 1995, therapy with at least a week of conventional amphotericin B resulted in a mean 44% response rate in pulmonary IA (52). In this infection, a dose of 1.0 to 1.5 mg/kg/day should be used despite the high incidence of nephrotoxicity. There is less experience with the lipid formulation of amphotericin B. While head-to-head randomized trials have not compared conventional and lipid preparations in IA, comparison to historic controls shows lipid formulations to be similar in efficacy. In comparative trials in patients with neutropenic fever (most of whom do not have IA), the lipid preparations provide a modest reduction in nephrotoxicity (19% vs. 34% in one study) at an enormously increased cost (76, 77).

Until recently, the only other therapeutic option for IA was itraconazole. Itraconazole, a triazole antibiotic, also interferes with the fungal cell membrane, in this case by inhibiting a fungal P450 enzyme responsible for ergosterol synthesis. This results in accumulation of an ergosterol precursor, lanosterol, in the membrane and functional impairment of several important membrane proteins, causing a fungistatic (rather than fungicidal) effect. Itraconazole is much better tolerated than amphotericin B and is active against *Aspergillus* species in vitro. In open-label observational studies, patients with IA had a complete or partial response rate 39-63% to itraconazole therapy (78, 79), although head-to-head comparison with amphotericin has not been performed. Several important considerations dampen enthusiasm for itraconazole in IA, however: 1) highly variable serum levels depending on patients' P450 genotype, 2) complicated drug interactions with many medications through its effects on human P450 enzymes, and 3) poor absorption of capsule preparations.

Last year the FDA approved caspofungin for IA in patients who have either failed or cannot tolerate other forms of therapy. Caspofungin is the first member of a new class of antifungal agents, the echinocandins, which target the β 1,3-d-glucans of the hyphal wall of many fungi. The drug is fungicidal against *Aspergillus* and *Candida* species in vitro, and is relatively well tolerated. Remarkably, there are no published trials of this drug for IA; the FDA approval was based on an unpublished, company-sponsored, open-label, non-comparative study of 63 patients, in whom 41% had a complete or partial response (data from an abstract, Maarten J, ICAAC 2000 40:371, abstract #1103). The data in support of the use of this medication must, therefore, be taken on faith.

An investigational triazole antibiotic, voriconazole, is close to approval by the FDA. Its mechanism of action is similar to itraconazole, and its in vitro range includes *Aspergillus* species and some other invasive molds. In a published observational open-label study, 41% of 116 patients with IA had a complete or partial response to the drug (80). Lastly, early surgical resection of infected tissue is strongly advocated by some authorities if the lesion is focal and the host can withstand the procedure (81).

D. Invasive infection and the effector phase of innate immunity:

It is fairly clear that the majority of patients develop IA due to major defects in the effector phase of innate immunity. Therapy aimed at augmenting this immune response to the pathogen, therefore, seems logical. Examples of such strategies include administration of colony stimulating factors and leukocyte transfusions in neutropenic patients with IA. The best clinical data for immunomodulation in IA is for interferon-γ (IFN-γ) therapy in children with chronic granulomatous disease (CGD). CGD patients have one of several inherited defects in the components of NADPH oxidase, resulting in impairment of superoxide anion production by innate effector cells (82). As a result, these children suffer from recurrent bacterial and fungal infections. In large series that predate IFN-γ therapy, the incidence of IA was 16% in this disease (83). Both in vitro and in animal models on invasive aspergillosis, exogenous IFN-γ improves the clearance of the pathogen (84-86). In a randomized controlled trial in 1991, CGD patients treated prophylactically with IFN-γ three times weekly had half the rate of infection compared to placebo (87), associated with improved neutrophil-mediated hyphal killing ex vivo (88). Whether IFN-γ therapy is effective in treatment of patients with IA is only supported anecdotally by case-reports.

Another approach might be to augment the chemotaxis of the available innate effector cells to the site of infection. Chemokines, a group of structurally related cytokines, were originally described on the basis of their chemotactic activity for various classes of leukocytes. A subset of chemokines (including human IL-8) contain the ELR amino-acid sequence near their N-terminus and are strongly chemotactic for neutrophils. This group of chemokines are expressed in response to exposure to *Aspergillus* by human cells in vitro, by respiratory epithelial cells in vivo, and in animal models of the infection (89-92). The common receptor for these ligands in the mouse is CXCR2, and blocking this receptor renders healthy mice (which normally clear *Aspergillus* conidia from the lungs without developing disease) extremely susceptible to invasive aspergillosis (Figure 6), associated with greatly reduced influx of neutrophils into the lungs (93). Conversely, transient over-expression of one these ligands in the lungs of immunocompromised mice with IA resulted in increased neutrophil influx, improved fungal clearance, and greater survival. These data support a potential therapeutic role for these molecules in IA.

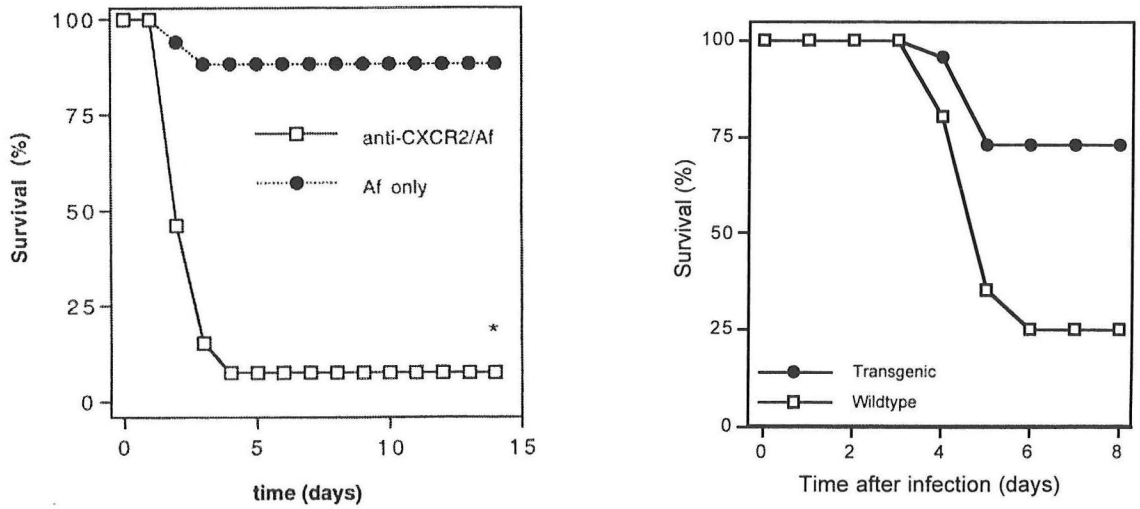

Figure 6. Survival in mice challenged with intrapulmonary *Aspergillus* conidia. Immunocompetent mice with receptor blockade (left, from 93); immunocompromised mice with transgenic over-expression of ligand (right, manuscript submitted).

Hypersensitivity diseases

A. Clinical review:

Aberrant host immune response to *Aspergillus* can result in hypersensitivity pneumonitis, asthma, or allergic bronchopulmonary aspergillosis (ABPA). Hypersensitivity pneumonitis (HP, or extrinsic allergic alveolitis) is a disease characterized by an abnormal Th-1 host response to any of variety of antigens (94). HP due to *Aspergillus* species was originally described in the so called malt worker's lung, due to exposure to moldy barley (95, 96) but also occurs due to exposure to moldy hay, compost, in greenhouses, and tobacco leaves (97-101). As in other forms of HP, there is a spectrum of presentation from an acute disease characterized by fever, dyspnoea, and bilateral infiltrates, to a chronic illness characterized by dyspnoea and cough associated with fibrosis and honey-combing of the lungs. Consistent with the Th-1 mechanism, nearly all of the patients have IgG, but not IgE, to *Aspergillus* antigens.

Th-2 hypersensitivity to molds in general, and to *Aspergillus* species in particular, is strongly associated with asthma: 15-25% of asthmatics have immediate (IgE-mediated) skin hypersensitivity to *Aspergillus* antigens, and 10% have serum precipitating antibodies to the organism (102-104). In addition, the severity of asthma symptoms and frequency of asthma ER visits correlate with environmental fungal spores (105-107). This obviously does not prove causation, but is a suggestive association.

Allergic bronchopulmonary aspergillosis (ABPA) is the best characterized allergic disease to *Aspergillus* species. It occurs in 1-2% of asthmatic patients and 7-35% of patients with cystic fibrosis (108-111). The disease is characterized by colonization of airways with *Aspergillus* hyphae (rarely other molds), asthma of varied severity, exacerbations consisting of worsening cough and sputum production in association with alveolar infiltrates, and progression to bronchiectasis and end-stage lung disease in a proportion of patients. Diagnosis is difficult and is based on a constellation of clinical features (Table 5). These criteria have not been validated prospectively in reference to a gold-standard, and criteria used by different investigators differ somewhat. In addition, *Aspergillus* antigens used for skin testing are not standardized (112, 113).

Diagnostic criteria for ABPA
Essential:
1. Asthma or cystic fibrosis
2. Immediate skin hypersensitivity to *Aspergillus* antigens
3. Total IgE >1000 ng/ml
4. Elevated *Aspergillus*-specific IgE and IgG by ELISA (IgG can also be detected as serum precipitating antibodies)
5. Peripheral blood eosinophilia
Confirmatory:
1. Infiltrates on chest radiogram
2. Proximal bronchiectasis
3. Isolation of *Aspergillus* species from sputum
4. Expectoration of brown plugs
5. Delayed (Arthus-type) skin hypersensitivity to *Aspergillus* antigens

Table 5. Adapted from (103, 114). Some authors do not consider peripheral eosinophilia an essential criterion (115)

It is helpful to think of the disease in terms of clinical stages (Table 6), but, similar to the stages in sarcoidosis, patients do not necessarily progress from one stage to the next. Chest radiographs show patchy alveolar infiltrates with a predilection for the upper lobes during acute exacerbations. Segmental or lobar atelectasis can develop due to mucus plugging. Later stages of disease are characterized by evidence of bronchiectasis involving the proximal airways. Mucus plugs in these branching proximal bronchiectatic airways are sometimes visible radiographically, and are referred to as "finger-in-glove" or "toothpaste shadows."

Stage		Presentation
I.	Acute	Fever, wheeze, cough, infiltrates
II.	Remission	Asymptomatic, normal CXR
III.	Exacerbation	Fever, wheeze, cough, infiltrates
IV.	Corticosteroid dependent asthma	chronic wheezing, no infiltrates
V.	End-stage Lung disease	Honeycombing, bronchiectasis

Table 6. Clinical stages of ABPA, originally proposed by (116)

ABPA is a clinical diagnosis and most patients do not undergo a lung biopsy. Two related conditions, mucoid impaction of bronchi (MIB) and bronchocentric granulomatosis (BG), are histopathologic entities with substantial overlap with ABPA. Specifically, 80% of patients with MIB and half of those with BG have asthma, atopy, tissue or blood eosinophilia, and airway colonization (but not tissue invasion) with *Aspergillus* species. In these patients, BG and MIB probably represent the histopathologic counterpart of clinical ABPA. In cases of MIB and BG without asthma or eosinophilia, the etiology is unclear.

Treatment of ABPA centers on the use of systemic corticosteroids, which results in lowering of IgE levels and peripheral eosinophilia and clearing of infiltrates. A widely used regimen recommends starting with prednisone 0.5mg/kg/day with gradual taper over 3 months, while serially monitoring total serum IgE levels (117). Two recent systematic reviews by the Cochrane group found that therapy of asthmatic patients with ABPA with itraconazole resulted in fewer exacerbations and reduced serum IgE and sputum eosinophils, but there was insufficient data in patients with cystic fibrosis (118, 119).

B. Host defense in allergic bronchopulmonary aspergillosis:

If we accept that at least some of the 25% of asthmatic patients with evidence of sensitization to *Aspergillus* antigens develop worsening symptoms after inhaling the conidia, why do they not develop ABPA? A key differentiating feature between the two diseases is the colonization of the airways of ABPA patients with the *Aspergillus* hyphae, whereas asthmatics respond only to inhaled conidia, which are cleared normally and do not grow into hyphae in the airways. There are some differences in the antigens extracted from conidia and hyphal forms of the organism (120, 121). Is there any data to support the idea that the acquired host response to the conidia differs from the response to hyphae? In a study in mice, lung dendritic cells phagocytosed both *Aspergillus* conidia and hyphae, but cells exposed to conidia produced IL-12, whereas those exposed to hyphae generated IL-4 (122), indicating that these antigen-presenting cells may be priming CD4 T-cells to develop into Th-1 and Th-2 phenotypes, respectively. To corroborate this, conidia and hyphal fragments were introduced into the lungs, and spleen and lymph-node CD4 T cells were assayed for IL-12 and IL-4 production. As depicted in Figure 7, CD4 cells from conidia-challenged mice produced IL-12 (Th-1 phenotype), and those from hyphae-challenged animals produced IL-4 (Th-2).

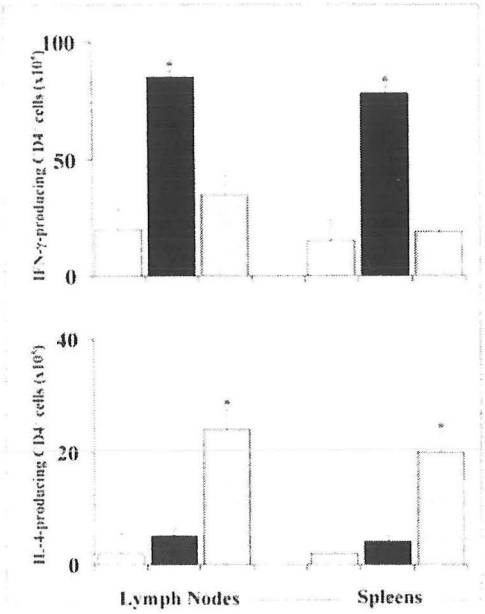

Figure 7. CD4 T cell in mice challenged with conidia (black bars), hyphae (gray bars) and controls (white bars). From (122).

If the hyphal forms of the pathogen is particularly likely to induce a Th-2 response, can elements of surface immunity be deployed to inhibit their formation? Lung surfactant proteins A and D (SpA and SpD) are members of a family of antimicrobial surface peptides called the collectins. The collectins are examples of the pattern-recognition molecules discussed earlier: they are small secreted proteins that recognize carbohydrate structures on the surface of diverse pathogens, including fungi. Pathogens bound by collectins have decreased virulence and are phagocytosed and killed by macrophages and neutrophils more readily (reviewed in 123). In vitro, SpA and SpD bind *Aspergillus* conidia and promote its phagocytosis and killing by human phagocytes (124-126). In addition, these molecules prevent the binding of IgE to *Aspergillus* allergens, and thereby inhibit IgE-mediated histamine release from basophils (127). Do these in vitro findings affect the in vivo course of ABPA?

In a mouse model of ABPA that shares many of the immunologic features of human disease, delivery of either SpA or SpD after established hypersensitivity resulted in decreased serum *Aspergillus*-specific IgE, peripheral blood eosinophilia, and lung eosinophil infiltration (128). In addition, SpA and SpD-treated animals had decreased production of IL-4 and IL-5 by their splenocytes (Figure 8). This is evidence in favor of a potential therapeutic role for these molecules in this disease.

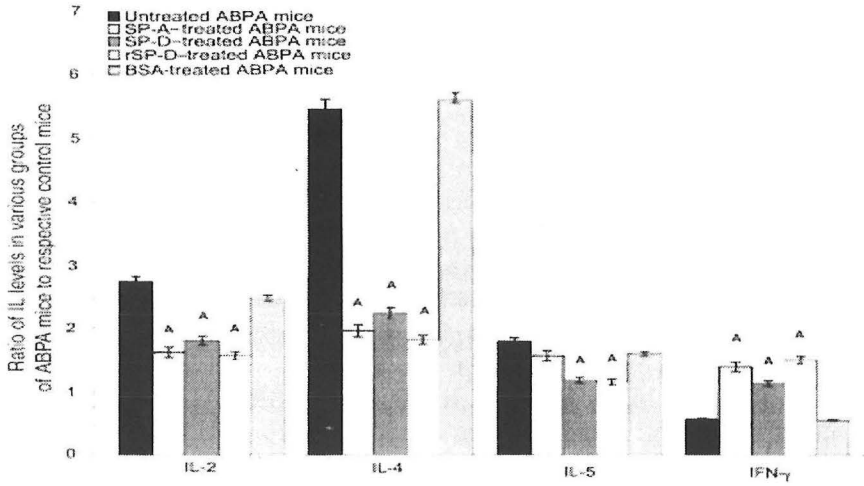

Figure 8. Spleen supernatant cytokine levels in ABPA mice treated with surfactant protein. From (128).

Conclusion:

Aspergillus species are unique in the diversity of human diseases they cause. There is good evidence that this diversity is, in large part, the result of the host response to the pathogen. Clinically, invasive aspergillosis is the most common and devastating of these illnesses; much work needs to be done to improve our detection, therapy, and prevention of this disease.

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