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Invasive aspergillosis in glucocorticoid-treated patients

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Glucocorticoids have potent, pleiotropic effects on the immune system that can predispose patients to developing life-threatening invasive aspergillosis (IA). While the exact prevalence and attributable mortality of IA in glucocorticoid-treated patients is difficult to estimate, *Aspergillus* species are significant pathogens in patients that require prolonged high-dose glucocorticoid therapy including multiple myeloma, collagen vascular diseases, or recipients of solid organ/hematopoietic transplantation. Experimental animal models and autopsy series have revealed important differences in the pathology of aspergillosis between glucocorticoid-treated and neutropenic patients. Although neutropenic hosts develop infection characterized by extensive angioinvasion, hemorrhagic thrombosis and necrosis with a high fungal burden, glucocorticoid-immunosuppressed hosts present with infection dominated by extensive necrosis, less angioinvasion, and a lower fungal burden suggestive of an inflammation-driven pathology. These pathobiological differences may have important implications for the diagnosis and treatment approaches of IA in glucocorticoid-treated patients.

Keywords Corticosteroids, aspergillosis, inflammation, immunity

Introduction

Glucocorticoids are potent immunosuppressive drugs that have been used for over 60 years in patients with autoimmune and allergic diseases, lymphoid malignancies, or solid organ/hematopoietic stem cell transplantation. The immunosuppressive activity of glucocorticoids stems from the pleiotropic effect of the glucocorticoid receptor blockade on signaling pathways involved in cytokine and eicosanoid production, cell adhesion, chemotaxis, antigen presentation and recognition, phagocytosis and immune cell survival and/or proliferation [1,2]. Because many of these pathways are essential for a coordinated and effective host response against *Aspergillus* species [3], patients who receive prolonged, high-dose glucocorticoid therapy are among the highest risk groups for death due to invasive aspergillosis (IA) [3].

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Glucocorticoid receptors further interact with a number of pathways critical for anabolic processes in integument, musculoskeletal system, and the vascular endothelium [3]. Interference with these pathways enhances susceptibility to fungal invasion and limits capacity of the host for tissue repair [2]. In addition, hyperglycemia, endocrine and electrolyte abnormalities, and poor nutritional status (suppressed appetite/cachexia) are frequently encountered in patients on high-dose chronic glucocorticoid therapy, further compounding already weakened immune responses against *Aspergillus* [2]. Because most of these anti-anabolic and immunologic effects are cumulative, some experts have proposed glucocorticoids to be the 'high-interest credit cards' of immunosuppressive therapy – drugs with immediate benefit but compounding future costs in terms of infections and side effects for the patients [4].

Historically, treatment guidelines for IA have not differentiated the management strategies based upon the underlying immunosuppression of the patient. The *2008 Treatment Guidelines for Aspergillosis* from Infectious Diseases Society of America provides the similar recommendations for management of IA in neutropenic and glucocorticoid-immunosuppressed patients [5].

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Experimental models and autopsy studies, however, suggest that the patterns of tissue injury, inflammation and repair differ substantially between patients with invasive pulmonary aspergillosis (IPA) in the neutropenic versus the glucocorticoid-treated, non-neutropenic background [6,7]. These pathobiological differences may have important implications for both the diagnosis and treatment of IPA in the glucocorticoid-treated patients. Herein, we review recent data concerning IPA in the non-neutropenic, glucocorticoid-immunosuppressed host and discuss the potential implications for diagnosis and management of the infection.

Immunopathogenesis of IA in the non-neutropenic, glucocorticoid-immunosuppressed host

Glucocorticoids affect multiple leukocyte cell lines, including T and B cells, macrophages, granulocytes, and monocytes [3]. When not bound to serum proteins, glucocorticoids cross cell membranes where they bind to glucocorticoid receptors (GR) in the cytoplasm and influence transcription in a positive or negative manner [2]. Therefore, there are some differences regarding the dominant action of glucocorticoids in each subset of immune cells. In lymphocytes and monocytes, glucocorticoids exert their negative effect on cytokine gene expression by directly inhibiting two transcription factors: activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) [8]. Glucocorticoid-inhibition of interactions between AP-1 and transcriptional regulatory proteins of the c-Fos/c-Jun family block IL-2 production and limit T-cell activation and proliferation [2]. Inhibition of NF-κB decreases the expression of several immunoregulatory genes, including interleukins -1, -2, -3, -4, -6, -10; interferon-gamma (INF-γ); CD40 ligand, tumor necrosis factor-alpha (TNF-α); granulocyte-macrophage colony stimulating factor (GM-CSF); leukotrienes, and expression of major histocompatibility complex molecules [2]. Glucocorticoids also inhibit other sites in T-cell activation pathway by blocking the decay of mRNA of cytokine-inducing genes and by inhibiting the tyrosine phosphorylation of intracellular proteins [8]. Glucocorticoids cause a transient, but significant, lymphocytopenia through redistribution of circulating lymphocytes, altering expression of adhesion molecules, and causing lysis of immature T-cells [8]. The effects of glucocorticoids on B-cells are mediated in part by reduction T-helper cell interactions required for antibody production [2].

The functional effects of prolonged high-dose glucocorticoids on alveolar macrophages are critical, as these immune cells are the primary lines of the innate cellular defense against inhaled *Aspergillus* conida [9–11]. In addition to potent suppression of NF-κB transcription, glucocorticoids inhibit both oxidative (NADPH-dependent) and non-oxidative mechanisms important for damaging *Aspergillus* conidia and hyphae [12]. Even low concentrations of glucocorticoids reduce healthy human neutrophil-mediated hyphal damage by $>20\%$ after as little as 10 minutes of exposure (Fig. 1). Presumably, this rapid onset occurs through pathways involving membrane-associated receptors and second messengers [2]. Extended incubation (>120 minutes) of dexamethasone with healthy-donor PMN decreases production of both pro-inflammatory (TNF-α, IFN-γ) and anti-inflammatory (IL-10) cytokines, and practically abolishes PMN-mediated damage of *Aspergillus* hyphae [13]. Neutrophil function can be restored, however, with stimulation of microbial pattern recognition receptors (PRRs), including toll-like receptors 2, 4, and 9; highlighting the plasticity of even glucocorticoid-suppressed cells [13].

Glucocorticoids can act as a double-edge sword in the homeostasis of inflammation induction and resolution [14–16]. While NF-κB activation in neutrophils at the onset of infection is required for pro-inflammatory gene expression, the activation of this transcription factor later in the inflammatory response is also critical for expression of genes controlling inflammation (i.e., IL-10), recruitment of Treg cells (IL-4), and induction of neutrophil apoptosis essential for orchestrating macrophages tissue repair (Fig. 2) [15]. Hence, high-dose glucocorticoid therapy may paradoxically contribute to prolongation of a dysregulated inflammatory processes in the lung predisposing the host excessive

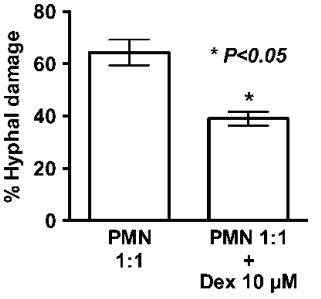

Fig. 1 Neutrophil-mediated damage of *Aspergillus fumigatus* hyphae is reduced within 10 min exposure to dexamethasone. Polymorphonuclear neutrophils (PMNs) were collected from a healthy volunteer and incubated in a 1:1 ratio with *A. fumigatus* hyphae $+/-$ 10 μM of dexamethasone (Dex). PMN were then hypotonically lysed and fungal hyphal viability was determined by reduction of 2,3-bis[2-methyloxy-4-nitro-5-[(sulfenylamino) carbonyl]-2*H*-tetrazolium-5-carboxanilide] XTT. Bars represent the mean% of hyphal damage calculated from non-PMN exposed controls $+/-$ standard deviation determined from an experiment performed in triplicate.

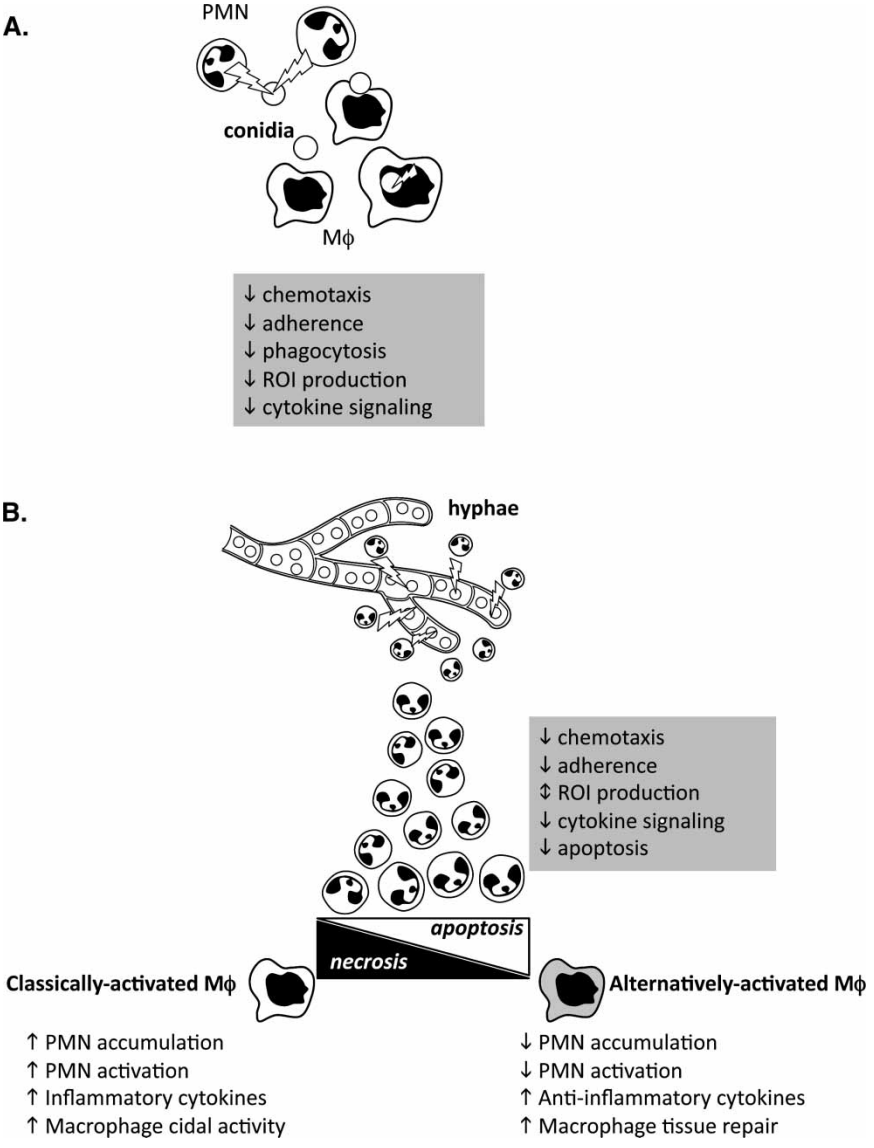

Fig. 2 Possible pathological mechanisms of glucocorticoids in the innate immune response to invasive pulmonary aspergillosis. Glucocorticoids impair a number of key effector functions in alveolar macrophages (Mϕ) and polymorphonuclear neutrophils (PMN) essential for killing of *Aspergillus* (A) conidia, and (B) hyphae. Inhibition of PMN apoptosis prolongs inflammatory pathology. Effects of glucocorticoids are presented in shaded boxes. ROI = reactive oxygen intermediates, Mϕ-macrophage, PMN-polymorphonuclear neutrophil.

tissue damage [14]. As glucocorticoids are potent inhibitors of neutrophil apoptosis [15], neutrophils may be functionally polarized to necrosis with *Aspergillus* exposure in glucocorticoid-associated inflammatory states. Persistent non-selective release of granules and reactive oxygen intermediate (ROI) in tissue would lead to excessive collateral damage to host tissue and impaired clearance of proliferating fungal cells [11,13,15,17].

Interestingly, some *Aspergillus* species exert direct deleterious immunomodulatory effects and contribute to a persistent deregulated inflammatory state in glucocorticoid-treated hosts through the release of secondary fungal metabolites. Specifically, gliotoxin, a member of the epipolythiodioxopiperazine (ETP) class of mycotoxins that is secreted in high concentrations by *A. fumigatus in vivo* [18,19], preferentially induces apoptosis in T-cells and monocytes over neutrophils [20,21]. Phorbol myristate acetate (PMA)-stimulated neutrophils incubated in the presence of gliotoxin and methyprednisolone *in vivo* release high concentrations of extracellular ROI, a marker of cellular necrosis, compared to stimulated PMNs without steroids exposed to gliotoxin [20]. This salutary mechanism of

gliotoxin ROI release and pro-inflammatory damage is suggested by data from animal models utilizing *A. fumigatus* mutants gliotoxin-encoding genes [22,23]. Deletion of the nonribosomal peptide synthetase GliP-encoding gene in *A. fumigatus* abolishes gliotoxin synthesis and is associated with attenuated virulence in glucocorticoid-immunosuppressed, but not neutropenic mice [22,23]. Similar studies using culture supernatants from *A. fumigatus* strains lacking a transcriptional regulator for gliotoxin synthesis ($\triangle$ *gliZ*), however, found a reduction in PMN apoptosis with impaired gliotoxin synthesis [24]. However, differences in apoptosis were only documented by the Annexin-V assay and virulence was not tested in a non-neutropenic animal model of IPA.

Animal models have been instrumental in dissecting differences in the immunopathology of glucocorticoid versus neutropenic IPA. Balloy and colleagues systematically evaluated patterns of fungal invasion and inflammation in glucocorticoid or neutropenic mice following intranasal challenge with *A. fumigatus* conidia [6]. A summary of their findings is presented in Table 1. The authors found that IPA pathogenesis in neutropenic animals was dominated by fungal proliferation, angioinvasion with necrosis with minimal inflammatory exudates. Both pro- and anti-inflammatory cytokines (TNF-α, IL-10) were still detectable in the bronchial alveolar lavage fluid (BALF), despite a lack of neutrophil influx. Fungal burden and galactomannan concentrations were high in neutropenic animals, and survival could be improved with amphotericin B therapy. In contrast, cortisone-immunosuppressed mice developed diffuse pneumonia after intranasal challenge with *Aspergillus* conidia that was associated with extensive neutrophil influx into the BALF, minimal TNF-α and IL-10 production, and limited fungal invasion with low galactomannan concentrations in the lung. Interestingly, amphotericin B was ineffective at prolonging survival in the cortisone-immunosuppressed mice. The findings suggest that, in contrast to the neutropenic background, the pathogenesis of acute IPA in the glucocorticoid-immunosuppressed mouse is propagated by an adverse host inflammatory response. In fact, control of this dysregulated inflammatory response has been shown in some models to be as critical as antifungal therapy for prolonging survival of glucocorticoid-immunosuppressed animals with acute IPA [25,26].

Despite the inherent limitations of animal models, immunopathological differences of glucocorticoid versus neutropenic IPA have striking similarities to histopathology of IPA observed in cancer patients with either neutropenia at the time of autopsy or glucocorticoid-treated graft-versus-host disease (GVHD) (Fig. 3) [27]. A recent review of autopsy-proven IPA at The University of Texas M.D. Anderson Cancer Center revealed that histopathology characterized by diffuse inflammation and limited *Aspergillus* hyphae was more common in allogeneic stem cell transplant recipients with GVHD who were treated with glucocorticoids (5/7) as compared to neutropenic patients (0/18) $P < 0.0004$ [27]. IPA in neutropenic patients was associated with minimal inflammation, a high burden of angioinvasive hyphae, and coagulative tissue necrosis. Unlike animal studies, each immunosuppression cohort had an equally high prevalence of disseminated infection- a likely reflection of differences in infection chronicity between animal models and patients [27,28].

Table 1 Immunosuppression-dependent patterns of fungal invasion and immunopathology in the glucocorticoid versus neutropenic experimental model of invasive pulmonary aspergillosis

	Glucocorticoids	Neutropenia
Cellular trafficking in the BALF	Rapid and extensive increase in PMN	No PMN influx
TNF-α concentrations in the BALF	Not detected	High
IL-10 concentrations in BALF	Low	High
Histological features	Diffuse and extensive consolidation and inflammation	Limited consolidation, necrosis with hyphae
Presence of fungal elements	Small numbers of conidia and poorly germinated hyphae	Extensive angioinvasive hyphae
Chitin concentrations in organs [fungal burden]	Low in all organs	High in all organs
Galactomannan concentrations in organs	Low to undetectable	High in all organs
Amphotericin B efficacy	No improvement in animal survival	Significant improvement in animal survival
Dominant mechanism	Adverse host inflammation	Unimpeded fungal growth and invasion

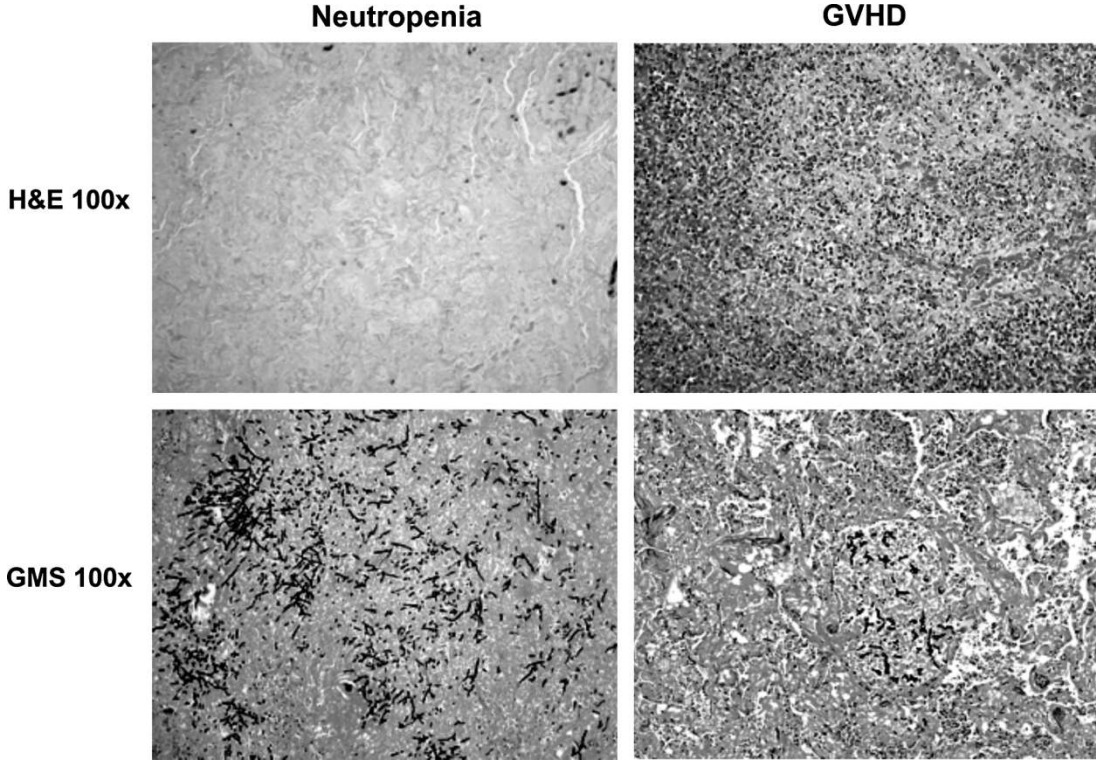

Fig. 3 Histopathology of invasive pulmonary aspergillosis in neutropenic versus allogeneic HSCT patients treated with high-dose glucocorticoids. H&E; Hematoxin and eosin stain. GMS; Grocott's methanamine silver stain. Neutropenic patients have minimal inflammation with high fungal burden and extensive angioinvasion and coagulative necrosis. In contrast, GVHD patients' extensive and severe inflammation primarily composed of PMN and minimal fungal burden.

The archetypical pathology of IPA in the glucocorticoid-immunosuppressed host may be more complex in highly immunosuppressed patients with underlying hematologic malignancy following transplantation compared to what is seen in the controlled setting of experimental models of aspergillosis. Combined types of immunosuppression (e.g., glucocorticosteroids and TNF inhibitors, sequential or combined glucocorticosteroids and neutropenia), concomitant bacterial (e.g., *Pseudomonas aeruginosa*) or viral (e.g., CMV) infections graft-versus host disease create more diverse and complicated immunobiology in patients. For example, in a small study Stergiopoulou and colleagues reported that the dominant histopathological finding of IPA non-neutropenic HSCT recipients with GVHD receiving glucocorticoids was acellular angioinvasive necrosis, suggestive of defective neutrophil trafficking to the lungs [29]. Hence, patients appeared to be functionally neutropenic in the lung allowing unimpeded proliferation of *Aspergillus,* despite normal or near-normal peripheral neutrophil counts. Further studies are needed to dissect the contribution of different elements contributing to the unique immunopathology patients who develop IPA in the setting treated of glucocorticoid treatment.

Nevertheless, these recent observations from human and experimental IPA collectively highlight notable differences in the pathology of IPA in the neutropenic versus glucocorticoid-immunosuppressed host and the importance of host-specific factors in tissue injury. The clinical implications of these differences have become more discernable with the growing frequency of late-onset IA in the allogeneic HSCT population, who receive prolonged glucocorticoid therapy in the post-transplant period for prevention and treatment of GVHD [28,30–32].

Clinical risk and presentation of IA in the glucocorticoid-immunosuppressed patient

Extent of glucocorticoid exposure

A number of studies have long shown that high-doses and prolonged treatment courses of glucocorticoids correlate both with increased risk and poor outcome of IA [3]. The most striking associations have been reported in the allogeneic HSCT population, where

high cumulative doses of glucocorticoids are administered to control acute or chronic GVHD. In an analysis of 331 allo-HSCT recipients, prednisone dosed at 0.5–1 mg/kg per day for GVHD was associated with a six-fold higher risk of IA compared to lower-dose regimens (0.25 mg/kg/day) [33]. Grow and coworkers showed that methylprednisone doses of at least 1 mg/kg administered for at least 21 days was associated with an increased risk of late IA and CMV after allogeneic HSCT [32]. Similarly, Marr and colleagues found that glucocorticoids administered late in the post allogeneic HSCT period increase the risk of IA in a dose-dependent fashion [34]. The risk of developing IA increases from 5% to 10% then 14% as prednisone/ methyprednisolone equivalent doses increased from 1.9 mg/kg/day to 1.9–3 mg/kg/day to greater than 3 mg/kg/day, respectively [34]. Furthermore, Ribaud and coworkers found that the risk of death increased significantly in allogeneic HSCT recipients with IA who received a cumulative prednisolone dose greater than 7 mg/kg in the weeks preceding infection compared to patients with doses of 7 mg/kg or less (20% vs 88% 60 day survival rate, respectively) [35].

Similar findings have been noted in expanding patient groups receiving high-dose glucocorticoid therapy, including patients with refractory multiple myeloma, solid organ transplant recipients, patients with collagen-vascular disorders, and acquired immune deficiency syndrome (AIDS) [36–41]. Gustafson and colleagues reported the risk of IA significantly increases in renal transplant patients with prednisone doses greater than 1 mg/kg/day [39]. Similarly, peri-operative glucocorticoid administration and boluses administered to prevent acute rejection were found to predispose patients to aspergillosis in liver, heart and lung transplant recipients [38]. None of these studies, however, identified a 'threshold dose' or duration of glucocorticoid therapy that could be used to identify highest-risk patients, i.e., candidates for *Aspergillus*-active antifungal prophylaxis [3]. The threshold level of glucocorticoid exposure probably differs from one patient group to the next because of concomitant immunosuppressive conditions or medications, co-infections (i.e., cytomegalovirus), period at risk for acquisition of IPA, differences in exposures, and innate immunity defects that further increase the risk of acquiring IA [3]. Finally, several cases of aspergillosis have also been reported in COPD or asthmatic patients receiving chronic, high-potency inhaled glucocorticoids [42,43], including infections involving the CNS [44]. These observations highlight the importance of considering aspergillosis as part of the differential diagnosis in any patient with a history of prolonged or high-dose glucocorticoid use.

Clinical presentation and diagnosis

Diagnosis of IA in the glucocorticoid-immunosuppressed patient is often delayed, because non-specific signs and symptoms of the infection, including fever, cough, pleuritic pain are blunted [3]. Glucocorticoids block the synthesis of prostaglandin E_2, the key mediator of temperature set-point in the hypothalamus, resulting in suppression of fever associated with early IPA [2]. Signs of fungal dissemination may also overlap with side effects of glucocorticoid therapy. For example, mental status changes due to central nervous dissemination of *Aspergillus* may be falsely attributed to the CNS side effects of high-dose glucocorticoid therapy [3].

High-resolution computer tomography scanning (CT scan) plays a critical role in the early detection of IPA [45,46]. Unlike neutropenic patients who frequently present with discrete macronodular lesions surrounded [initially] by ground glass opacity (halo sign) (Fig. 4) [47], glucocorticoid-immunosuppressed patients may initially present with bronchopneumonic forms of aspergillosis and more variable CT findings that includes multiple nodules, lobar infiltrates, or diffuse ground-glass opacities [48]. These peribronchial areas of consolidation and its radiographic manifestations are non-specific and indistinguishable from those caused by other pathogens [48]. Furthermore, the clinical course and natural progression of these lesions in glucocorticoid-immunosuppressed patients is often heterogeneous and heavily influenced by concomitant infections. In fact, concomitant infection by other pathogens associated with suppressed T-cell mediated immunity, including Pneumocystis [49], *Nocardia* [50], and atypical mycobacteria [51] are well-described in glucocorticoid-treated patients with aspergillosis. Hence, clinicians must have a high index of suspicion for *Aspergillus* infection in glucocorticoid-immunosuppressed patients even in the absence of 'classic' nodular infiltrates [48].

The yield of diagnostic methods such as histopathology and cultures in glucocorticoid-associated IPA is suboptimal reflecting the low fungal burden seen in this entity [52,53]. Conversely, the positive predictive value of a respiratory culture for *Aspergillus* is a function of the net immunosuppressive state of the patient and is not always synonymous to IPA in glucocorticoid-treated patients [52]. The positive predictive value of respiratory cultures for IA decreases from 77% in patients with hematological

malignancies to less than 58% in glucocorticoid-immunosuppressed patients [52]. Similarly, the sensitivity of the Aspergillus galactomannan test is lower in non-neutropenic glucocorticoid-immunosuppressed host ($\sim$50%) compared to neutropenic patients (>90%). This reduced sensitivity is probably explained, in part, by the lower degree of fungal burden and limited angioinvasion that is seen initially in patients with glucocorticoid-associated IA [27,53]. In addition, it has been hypothesized that neutrophils are capable of clearing galactomannan from the blood by mannose binding receptors [54]. The performance of PCR detection of Aspergillus DNA or RNA in neutropenic vs glucocorticoid-treated hosts, however has not been adequately studied to define their comparative performance in non-neutropenic, glucocorticoid immunosuppressed hosts.

The sensitivity of the galactomannan test can be improved in high-risk, non-neutropenic patients if the antigen test is performed on bronchial alveolar lavage fluid (BALF), rather than serum [55,56]. A recent prospective study of medical ICU patients with underlying risk factors for invasive aspergillosis found that in proven IA, the sensitivity of galactomannan was 88% in BALF compared to 42% in serum [55]. However, large prospective studies will be required to better define the clinical variables that affect assay performance and to determine the optimal methods of specimen processing.

Antifungal pharmacology in the setting of glucocorticoid-induced immunosuppression

Beyond their direct mechanisms of inhibiting fungal growth, systemic antifungal agents have been shown to have varying degrees of immunomodulatory activity that could be either beneficial or deleterious in the glucocorticoid-immunosuppressed host. Amphotericin B (AMB) has pronounced pro-inflammatory activity in polymorphonuclear and mononuclear cells that results in the release of inflammatory cytokines (TNF-α, IL-6, IL-1RA, IL-1β), chemokines (IL-8, MCP-1, MIP-1β), nitric oxide (NO), prostaglandins, ROI, and intracellular adhesion molecule ICAM-1 [57–61]. The pro-inflammatory effects of AMB are mediated through direct interactions with membrane-bound pattern recognition receptors Toll-like receptor 2 and CD-14 in immune cells, which activate MyD88-dependent signaling cascades ultimately leading to upregulation of genes encoding for inflammatory cytokines [61,62]. Clinically, the pro-inflammatory activity of amphotericin B-deoxycholate (AMB-d) is associated with fever, chills,

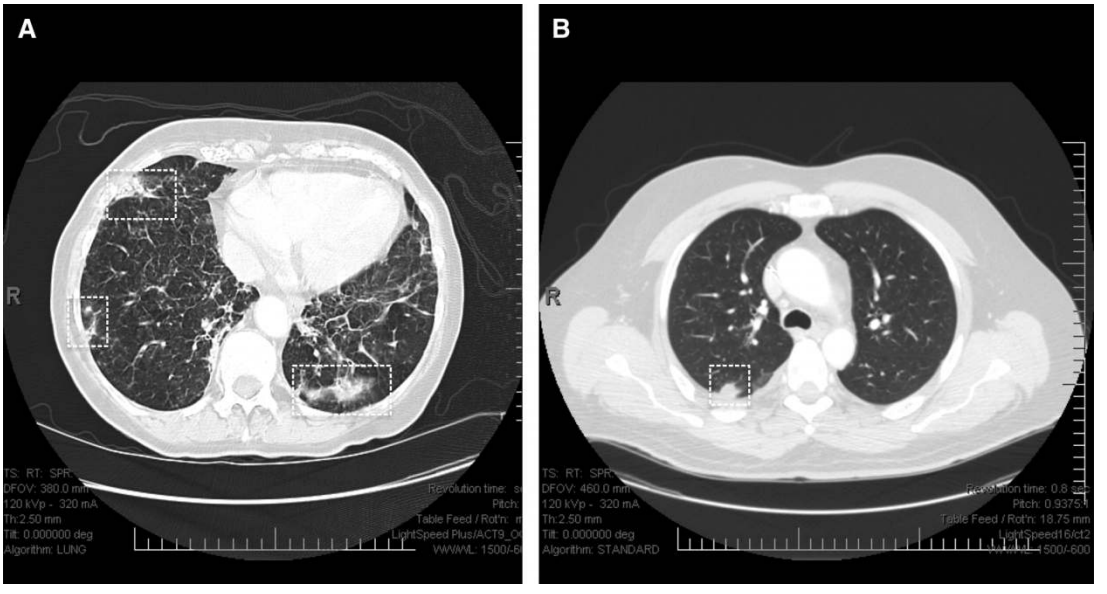

Fig. 4 Comparison of initial bronchopneumonic versus nodular presentation of patients with invasive pulmonary aspergillosis. Patient A is a 52-year-old female at Day +45 allogeneic HSCT for acute myelogenous leukemia with current ANC of 1800/μl and acute graft versus host disease receiving tacrolimus and 1 mg/kg/day methylprednisolone. Bronchoscopy cultures yielded *Aspergillus fumigatus* and *Pseudomonas aeruginosa*. The patient was afebrile at the time and serum galactomannan was negative. Patient B is a 50-year-old male with acute myelogenous leukemia on cycle 2, Day 27 of clofarbine and idarubicin chemotherapy with an ANC of 0. Patient has a fever of 38.7°C and complains of right sided pleuritic chest pain of two days duration. Serum galactomannan is positive at an index of 1.2.

myalgias and rigors during infusion of the antifungal that has been shown to correlate with increased plasma concentrations of TNF-α, IL-1RA, and IL-6 [63].

Theoretically, pro-inflammatory antifungals such as AMB-d would be expected to have potentially deleterious effects in the treatment of IA in the glucocorticoid-immunosuppressed host. This concern has been substantiated in animal models where AMB-d has shown limited efficacy in the prevention or treatment of IPA in the glucocorticoid-immunosuppressed animals [6,26]. Balloy *et al*. found that treatment with AMB-d reduced mortality in chemotherapy-treated (neutropenic) mice with IPA, but was ineffective in glucocorticoid-immunosuppressed mice [6]. Similarly, studies from our laboratories have demonstrated that while AMB-d significantly reduces *Aspergillus* lung fungal burden in neutropenic animals [26], the drug appeared to have minimal efficacy in the cortisone-immunosuppressed mice [26]. The impact of AMB pro-inflammatory activity in more chronic forms of non-neutropenic IA is less well understood but could be detrimental in chronic necrotizing forms of aspergillosis.

The pro-inflammatory activity of AMB changes in a formulation-dependent manner when the antifungal is incorporated into a lipid carrier [63–67]. Similar to AMB-d, AMB colloidal dispersion (ABCD) is associated with upregulation of inflammatory cytokine genes. In contrast, AMB lipid complex (ABLC) and liposomal amphotericin (L-AMB) either down-regulate or have minimal effects on cytokine production in macrophages and neutrophils [67]. These patterns of cytokine production appear to mirror the tendency of the formulations to cause less infusion-related toxicity, with the highest frequency of reactions elicited by AMB-d and ABCD, followed by ABLC and L-AMB [64].

Independent of their role as drug carrier, liposomes are known to have potent immunomodulating effects in phagocytic cells [68,69]. Bellochio and colleagues found that incorporation of AMB into liposomes diverts PMN TLR-2 activation to TLR-4, resulting in reduced production of TNF-α and ROI release and enhancement of non-oxidative killing mechanisms against *Aspergillus* hyphae [25]. We have recently shown that pre-treatment of glucocorticosteroid-immunosuppressed mice with empty liposomes reduces inflammatory pathology and improves fungal clearance and survival following intranasal inoculation of *A. fumigatus* [26]. Remarkably, the efficacy of empty liposomes in the glucocorticoid-immunosuppressed mouse model approached that of 10 mg/kg body weight of L-AMB, highlighting the impact of inflammation on antifungal pharmacology for aspergillosis in the glucocorticoid immunosuppressed host [26].

In contrast to AMB formulations, triazoles appear to have relatively few direct effects on mononuclear and polymorphonuclear phagocytes. While one study has suggested that voriconazole induces expression of TLR2 and TNF-α production in monocytes [70], most studies have shown that primary benefit of azoles lies in their direct inhibitory effects on fungal growth, locking pathogens into more favorable forms for host phagocytosis [71,72]. For example, triazole-associated depletion of ergosterol in the fungal cell membrane may further render cells more susceptible to host immune effector molecules, including ROI and NO [73,74]. Nonetheless, available data do not suggest major differences in the activity of voriconazole or posaconazole in neutropenic versus glucocorticoid-immunosuppressed host.

Echinocandins are the newest class of antifungals approved for the treatment of IA. The echinocandins uniquely target the fungal cell wall by blocking the synthesis of β-1,3-D glucan – a key structural polymer that is essential for filamentous growth in *Aspergillus* species [75]. Inhibition of glucan synthesis reduces bulk β-glucan levels in *Aspergillus* [76] that is most apparent at the apical tips, causing dysmorphic hyphal growth that is not globally lethal [77]. Despite this lack of *in vitro* fungicidal activity, echinocandins have proven to be effective at prolonging survival in animal models of aspergillosis [78–80], highlighting a discrepancy between *in vitro* susceptibility and *in vivo* efficacy that may be explained by immunomodulatory effects of the drug.

Recently, echinocandins have been reported to have mechanisms of immunostimulatory activity through direct or indirect actions on effector cells critical for control or clearance of *Aspergillus*. Pre-exposure of monocytes and macrophages in culture to echinocandins enhances the activity of these cells to damage *A. fumigatus* germlings that was greater than that of either the echinocandin or unexposed mononuclear cells alone [81]. On the other hand, pre-exposure of PMNs to caspofungin did not appear to affect their activity against *A. fumigatus* [81].

The immunostimulatory effects of the echinocandins seems to be linked, in part, to their ability to unmask immunogenic β-(1-3) epitopes on the surface of the fungal cell wall [82]. Beta glucans are recognized by PRRs expressed on alveolar macrophages, neutrophils, and dendritic cells [11]. The most important of these PRRs is Dectin-1, a NK cell receptor-like C-type lectin receptor that specifically recognizes β-(1,3) glucans of human pathogenic fungi and triggers inflammatory

responses that include the release of TNF-α and IL-12, neutrophil chemoattractants, and ROIs [83]. Interestingly, echinocandin exposure appears to alter immunogenic beta-glucan surface exposure differently in swollen conidia and germlings than in mature hyphae [84]. In swollen conidia and germlings, where β-glucans is highly surface exposed, echinocandin reduce surface glucan exposure and reduce Dectin-1 dependent macrophage inflammatory responses [84]. However, in drug treated hyphae of *Aspergillus* species and several other medically-important non-*Aspergillus* moulds (*Rhizopus oryzae, Fusarium solani, Fusarium oxysporum, Scedosporium apiospermum*), higher degrees of surface glucan exposure following echinocandin exposure were associated with enhanced neutrophil-mediated damage of hyphae, especially when neutrophils were incubated with a monoclonal antibody targeting β-glucan [85]. Currently, animals studies are underway to explore how antifungal immunomodulation affects the pathology of IPA in the glucocorticoid-immunosuppressed host.

Taken together, these pre-clinical studies support the concept that the type of underlying host immunosuppression has a profound effect on the pharmacology and efficacy of antifungal agents for IPA. Additional studies are needed to further explore how differences in host pathology between the neutropenic and glucocorticoid-immunosuppressed patient affect the activity and potential immunopathology of antifungal therapies used in the management of IPA.

Conclusion and perspective for future directions

Glucocorticoid-immunosuppressed patients develop a form of IA that is pathobiologically unique from neutropenic patients. As such, diagnostic strategies and treatment approaches need to be individualized to the immunosuppression of the host. Future clinical studies should stratify the performance of diagnostic tests and efficacy of antifungal agents in non-neutropenic glucocorticoid-treated versus neutropenic hosts with IA. It will also be important to better understand the impact and temporal effects of tapering of glucocorticoid therapy in the outcome of *Aspergillus* infection. Interpretation of clinical and animal studies could be vastly improved by development and validation of new biological markers that could be used to 'index' of the functional immunocompetence of glucocorticoid-treated hosts. Such markers could help better identify glucocorticoid-patients who are at higher risk for developing opportunistic infections, and help dissect the contribution of anti-anabolic and metabolic effects of glucocorticoids on the pathogenesis of IA.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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