Medical Mycology September 2006, **44**, S229–S235

Taylor & Francis
Taylor & Francis Group

Th1/Th2 in aspergillosis

DAVID A. STEVENS

Department of Medicine, Santa Clara Valley Medical Center and Stanford University Medical School, San Jose and Stanford, California, USA

The outcome of the Th1/Th2 balance is a critical determinant of the outcome in invasive aspergillosis. The innate immune system encounters the pathogen first. Dendritic cells (DC) appear to be the critical fulcrum at the intersection of the innate immune system, and which receptors are engaged, particularly which Toll-like receptors are triggered by the pathogen, likely determines which co-stimulatory molecules are expressed during the DC maturation process, and thus which cytokine pathway will eventually dominate. Some proinflammatory cytokines initially produced by naïve phagocytes may also direct dendritic cell direction. Thus DC are the main connections of the innate and adaptive immune systems. The cytokine pathway may be affected by the antigen load, whether and what type of immunosuppressive drug or immunosuppressive co-morbidity is present, the pathogen's success in converting from conidia to hyphae, the pathogen's production of toxins, and later whether appropriate antimicrobial chemotherapy is given. Chemokines triggered by cellular interaction with the pathogen call different host cell populations to the site of infection, collectins affect the phagocyte-fungus interaction, and the cytokines produced by the adaptive immune system then regulate the antifungal power of mononuclear phagocytes and polymorphonuclear neutrophils. In these ways the innate and adaptive immune systems work together in host defense. The key cytokines associated with a successful outcome of *Aspergillus* infection are upregulation of Th1 and proinflammatory cytokines IFNγ, TNFα, IL-12, GM-CSF, IL-1, IL-6 and IL-18, and down-regulation of IL-4 and IL-10. The converse is associated with progressive disease. The most data about favorable cytokine effects on phagocytes vs. *Aspergillus* concerns IFNγ, GM-CSF and G-CSF, and for unfavorable effects, IL-10 and IL-4. GM-CSF and IFNγ have also been shown to possess the ability to reverse the down-regulating effects of immunosuppressants on anti-*Aspergillus* phagocyte function. Neutralization of several Th1 cytokines *in vivo* has been shown directly to result in a bad outcome of infection, whereas administering Th1 or proinflammatory cytokines or neutralizing Th2 cytokines has been shown to produce a favorable outcome. Antifungal chemotherapy is associated with a switch to a Th1 profile, and antifungal chemotherapy combined with Th1 cytokine immunotherapy acts synergistically. Thus improved definition of the Th1/Th2 balance is essential for future prospects for immunotherapy, antimicrobial chemotherapy, and vaccination.

Keywords aspergillosis immunology, cytokines, aspergillosis host defenses, immunology, host response

Correspondence: David A. Stevens, Department of Medicine, Santa Clara Valley Medical Center, 751 S. Bascom Avenue, San Jose, CA 95128-2699, USA. Tel: +1 408 885 4302; fax: +1 408 885 4306; E-mail stevens@stanford.edu

DOI: 10.1080/13693780600760773

Introduction

The Th1/Th2 balance has been understood for some time as the critical determinant of outcome of the infection with *Aspergillus*. The focus of this review shall be invasive aspergillosis, particularly the pulmonary form, as the deflection into the Th2 pathway that results in asthma or allergic bronchopulmonary aspergillosis is being reviewed elsewhere in this symposium and has been reviewed in the prior supplement [1,2], and the relevant literature was exhaustively cited and discussed in the first supplement [3].

The Th2 pathway and allergy

In brief [3], the immune responder to *Aspergillus* antigens in allergic bronchopulmonary aspergillosis (ABPA), allergic asthma, and cystic fibrosis (CF) is a Th2 CD4+ cell. How does ABPA differ from the others? The hypothesis is ABPA develops in genetically susceptible asthmatics or CF patients.

In ABPA, *Aspergillus fumigatus* attaches to, grows in, and disrupts airway epithelium; and is not killed by mononuclear and eosinophil infiltrates. The proteolytic enzymes of *A. fumigatus* may be important in the epithelial interaction. The degraded epithelium fails to produce mucus that protects against particulates (such as spores). The degraded epithelium facilitates transport of antigens and allergens, leading to a Th2 response. Pro-inflammatory cytokines are also triggered. *Aspergillus* attaches to the extracellular matrix of the damaged airways, continuing the inflammatory cycle. IgE binds *Aspergillus* antigens to mast cells; mast cells release histamine and chemokines for eosinophils. IgE, IgA, and IgG bind *Aspergillus* antigens to eosinophils, which release inflammatory mediators. Epithelial junctions are disturbed, and airways are remodeled.

ABPA, compared to other atopic diseases, appears to have increased sensitivity to IL-4 from Th2 CD4+ cells, with increased B cell CD23 and CD86 expression, and increased IgE production. ABPA patients react to Asp f 2, Asp f 4, Asp f 6 antigens; asthmatics without ABPA to Asp f 1, Asp f 3. The differences may be genetically programmed. Patients can be identified by their reaction profiles. Certain HLA-DR genotypes are associated with reactivity to *Aspergillus* antigens.

Invasive aspergillosis

The normal initial response and its perturbation

The normal host requires an intact cell-mediated immune system and a sufficient number of, and normally functioning, neutrophils to effect the normal response to *Aspergillus* conidia, which we all continually encounter in the environment. This normal response is to clear conidia without establishment of an infectious nidus in the lung or dissemination to other organs. That a weakened cell-mediated immune system or insufficient neutrophils can cope with a few conidia but not a larger number is indicated by dose-response challenge studies in animal models and suggested by air filtration studies in immunocompromised patient units.

This normal response appears to be determined by encounters with mononuclear phagocytes. The first immunological line of defense against *A. fumigatus* is the macrophage, which is capable of ingesting and lysing conidia [4]. Both monocyte-derived and resident macrophages contribute to conidial ingestion. Killing is done by an opsonin-independent non-oxidative method. This line of defense is depressed by glucocorticoids [5–10]; not via steroid depression of T cells, but possibly by causing suboptimal phagolysosomal fusion, or through inhibition of non-oxidative processes and impairment of lysosomal activity, plus an effect on cytokine production. The second line of defense, once conidia have germinated, is neutrophil and monocyte killing of hyphae. This can occur in the absence of opsonins or activation. Both oxidative mechanisms and defensins appear to be involved. Monocytes principally use hydrogen peroxide. Steroids suppress this line of defense, probably in part by their suppression of the oxidative burst and possibly by decreasing cellular mobilization [11].

Networking of the innate immune system

The mononuclear phagocytes respond with pro-inflammatory cytokines, and different Th cytokines to different morphologic forms of *Aspergillus*, and can provide signals that further affect phagocyte antifungal activity, in an autocrine fashion, and that of other local phagocytes, in a favorable manner. Key defensive cytokines against conidia appear to be IFNγ and TNFα. Collectins, including mannose-binding lectin, SP-A, SP-D, and pentraxin 3 may help phagocytes by acting as opsonins, and another branch of the innate immune system, the production of chemokines, may call on the appearance of other phagocytes at the local site [12,13]. If, because of insufficiencies in phagocyte number or function, a more durable response is called for, the innate immune system will become further engaged and initiate the engagement of the adaptive immune system.

The fungus is recognized by Toll-like receptors (TLR) [14–18], and this encounter in dendritic cells

appears to be the critical one for the subsequent coordination of the arms of the immune system. If the conidial intruders have not been contained by phagocytes, and some have germinated into hyphae, the latter morphologic form may subvert the development of the appropriate response. The attachment of these forms to different dendritic cell receptors, probably mannose receptors in the case of conidia and CR3 in the case of hyphae [19], and the different methods of dendritic cell phagocytosis of the different fungal forms, may lead to different dendritic cell signals and thus to different cytokine patterns [20].

The dendritic cells take the internalized fungal forms, with the fungal antigens exposed or released, and migrate to regional lymph nodes. There the dendritic cells mature and express MHC II molecules, and process the antigen, and the TLR pathway triggered [21] likely determines which co-stimulatory molecules are expressed, and thus whether Th1 or Th2 cytokine pathways ensue. The cytokines initially produced by the naïve phagocytes previously discussed may also direct the dendritic cell direction [22]. Regulatory T cells may play a late role in directing this phase of the immune response at this point, inhibiting the development of allergy [23]. The adaptive immune system, represented by T cells, is then engaged, and the T cells, in the context of MHC molecules, become activated and proceed to elaborate either Th1- or Th2-dominant cytokines, thus determining the adaptive response.

The adaptive response – Th1

Clinical studies indicate IFNγ levels are increased and IL-10 levels decreased in survivors of infection [24]. Patients responding to therapy have high IFNγ/IL-10 ratios and strong Th1 lymphoproliferative responses [25,26].

The key cytokines associated with a successful response to *Aspergillus* infection are the pro-inflammatory and Th1 cytokines IFNγ, TNFα, IL-12, GM-CSF, IL-1, IL-6, and IL-18 [12,22,26–35]. Their production has been demonstrated in the course of infection, and this production, rather than the down-regulation of Th2 cytokines IL-4 and IL-10, appears to be even more important [36].

A great deal of evidence has been produced to stress the critical importance of Th1 and Th2 cytokines in the outcome of infection.

Mononuclear cells produce IFNγ, GM-CSF, TNFα and IL-2 in response to conidia [30,37]. Studies suggest that although the Th1-stimulating antigens are in conidia, hyphal components favor the Th2 path for both human and murine cells [38].

Although early studies indicated nude mice (lacking T cells) were not more susceptible [39], it's unclear whether the enhanced macrophage and NK activity in nude mice, or the severity of the infection studied (thus overwhelming wild-type mice), may have contributed to the conclusion that T cells are unimportant. Passive transfer of Th1 cells is protective [34].

TNFα deficient animals can't control the early phases of infection [26,40,41], neutralization of TNFα with antibody reduces neutrophil influx into the lung [31], and exacerbates the infection [33], and administering TNFα, such as intratracheally, enhances survival [26,28,32].

Neutralization of IFNγ exacerbates disease [24] and giving IFNγ is protective [26,28,34]. IFNγ enhances neutrophils damage to hyphae [24], and reverses the depressing effect of corticosteroids on monocyte [5] or neutrophil [27] anti-hyphal activity. IFNγ upregulates neutrophil damage of hyphae even with CGD neutrophils [30]. Administration of IFNγ to infected mice is associated with low IL-10 levels, and neutralization of IFNγ with elevated IL-10 levels [28].

A delay in IL-6 production leads to a decrease in neutrophil influx [11], and IL-6 deficient mice are more susceptible [34]. Conidia may preferentially stimulate the IL-12 pathway of pulmonary dendritic cells [19]. Neutralization of IL-12 in mice exacerbates disease [33], and therapy with IL-12 increases resistance [40,41]. Neutralization of IL-18 enhances the disease [33].

GM-CSF reverses the depressive effects of steroids on monocyte anti-hyphal activity [5]. Our laboratory has studied the effect of dexamethasone [DEX] and granulocyte-macrophage colony-stimulating factor [GM-CSF] on the fungicidal activity of macrophages and cytokine/chemokine production from macrophages. GM-CSF protects peritoneal macrophages [PeM] and bronchoalveolar macrophages [BAM] against DEX suppression of killing of *A. fumigatus* conidia [7,8].

We showed murine BAM killed conidia (33%) *in vitro*, unaffected by GM-CSF [9]. DEX treatment for 48 h reduced killing to 13%. GM-CSF restored killing (33%) in the presence of DEX. GM-CSF protected even if added after DEX treatment, provided DEX was discontinued.

Likewise, PeM killed conidia, which was suppressed by DEX. Both recombinant murine and human GM-CSF were effective in reversing DEX suppression of either BAM or PeM. However, the recombinant human GM-CSF only reversed DEX suppression of PeM if it was given before or concurrently with DEX [8].

We found *A. fumigatus* conidia stimulated BAM *in vitro* to produce IL-1α and TNFα. GM-CSF didn't

affect this, but DEX suppressed it. The DEX suppression of IL-1 (but not TNF) was reversed by GM-CSF. GM-CSF enhanced BAM (but not PeM) IL-1 and TNF production in response to LPS. DEX suppressed BAM and PeM IL-1 production and BAM TNF production, and GM-CSF reversed the DEX effect. We also found PeM production of IL-10 was much greater in response to conidia (or LPS) than BAM [10].

We reported recombinant murine GM-CSF given *in vivo* increases spleen cell proliferative responses *ex vivo*, and spleen cell responses to concanavalin A (presumably by increasing IL-2). Murine GM-CSF given *in vivo* increases spleen cell IFNγ, and (with a delay) increased IL-10, production, in response to concanavalin A *ex vivo*. IL-4, however, was unaffected [42].

We also found conidia stimulate BAM for MIP-1α production *in vitro*. If DEX is given *in vivo*, BAM from those animals now have suppressed MIP-1, IL-1 and TNF production *ex vivo*. If GM-CSF was given *in vivo* together with DEX (or cortisone), BAM responses with respect to these cytokines and chemokines was normalized [6].

We postulated that DEX inhibition of NF-κB was opposed by induction of IκB kinases (IKK) by GM-CSF+conidia stimulation, degradation of IκB, and release of nuclear factor kappa B (NF-κB). We studied resident PeM from CD-1 mice and RAW 264.7 cells [43]. Cells were untreated or treated with DEX, GM-CSF or GM-CSF+DEX and corresponding cultures stimulated by conidia. Western blot analysis showed that in 2 h cytoplasmic extracts from DEX-treated cells had increased levels of IκB and nuclear extracts contained minimal NF-κB. GM-CSF decreased IκB levels in cytoplasmic extracts and increased NF-κB levels in nuclear extracts. In combined treatment, GM-CSF overcame the effect of DEX on cytoplasmic levels of IκB and resulted in increased nuclear levels of NF-κB. Conidia, apparently signaling through TLR-4, reduce cytoplasmic IκB and activate NF-κB. This pathway was blocked by DEX as evidenced by high levels of IκB in cytoplasmic extracts and minimal levels of NF-κB in the nucleus. GM-CSF decreased cytoplasmic IκB in conidia-stimulated cells and increased NF-κB in the nucleus. Blockade of conidia signaling by DEX was antagonized by GM-CSF as evidenced by decreased cytoplasmic levels of IκB, comparable to conidia alone, and increased NF-κB in the nucleus.

The adaptive response – Th2

In contrast, progressive infection is associated with enhanced production of IL-4, IL-10, IgE, and depressed IFNγ [41]. Hyphae may preferentially stimulate the IL-4 and IL-10 pathways [19].

Neutralizing IL-4 in mice enhances resistance [26,36,40]. Administering IL-4 enhances susceptibility [44]. Mice deficient in IL-4 are resistant, and they have increased IL-12 production and decreased IL-10 and IgE; if the IL-12 is neutralized, resistance is diminished, thus emphasizing the importance of IL-12 over the depression of IL-10 [36]. Administering soluble IL-4 receptor, a means of antagonizing IL-4, also results in resistance [26]. IL-4 depresses phagocyte damage to hyphae and the oxidative burst [30].

IL-10 also depresses monocyte anti-hyphal activity and the oxidative burst, and this can be reversed with IFNγ, GM-CSF or TGFβ. Administering IL-10 to mice enhances susceptibility [44]. Neutralizing IL-10 enhances resistance [36,40]. IL-10 deficient animals have increased inflammation at infection sites, and enhanced levels of Th1 cytokines [34], and we have shown the resistance of IL-10 gene knockout mice to aspergillosis [45]. IL-10 levels are high in neutropenic patients with aspergillosis [20,46].

However, IL-10 may have a favorable regulatory role in the response to *Aspergillus*, dampening harmful inflammation [23].

Corticosteroids

Corticosteroids have long been identified as a risk factor for human invasive aspergillosis. Corticosteroids have direct effects on the phagocytes [5–11,27], as described above. However, in addition, cortisone administration results in a delay in cytokine production, prevents monocyte recruitment to the foci of infection [11], depresses TNFα production [47] and depresses IL-12 mRNA [28]. To counter this, the favorable effect of IFNγ and GM-CSF in reversing the phagocyte-depressing effects of corticosteroids [5–11,27] on monocytes, polymorphonuclears and macrophages has already been noted.

Antifungal chemotherapy

Antifungal chemotherapy and cytokine immunomodulation act synergistically *in vitro*, and *in vivo* therapy of experimental mycoses, as extensively reviewed elsewhere [48,49]. In aspergillosis, amphotericin B (AmB) therapy has been associated with a switch to a Th1 profile, with enhanced IFNγ and depressed IL-4 in the serum [50]. Liposomal AmB may change signaling by *Aspergillus* from TLR-2 to TLR-4, attenuating the pro-inflammatory effects of AmB, and optimizing efficacy [51].

Prophylaxis with human recombinant granulocyte colony-stimulating factor (G-CSF) with AmB or itraconazole therapy showed some additive effect in neutropenic animal models of invasive aspergillosis, but not in those immunosuppressed with cortisone, which has a greater effect against macrophages. In a neutropenic murine model, human G-CSF alone was ineffective but with AmB showed synergy in survival greater than with itraconazole [52]. G-CSF administered to human volunteers increased the fungicidal activity against *Aspergillus* conidia through enhanced respiratory bursts of their PMNs by 4-fold [53]. However, there is no clear evidence G-CSF benefits patients with aspergillosis. One review found no significant reduction in fungal infections in acute myelocytic leukemia patients treated with G-CSF [54]. GM-CSF and macrophage colony-stimulating factor (M-CSF) treated human macrophages exhibit enhanced conidial phagocytosis, oxygen radical production, and hyphal damage [55,56]. There are case reports of GM-CSF as part of a treatment regimen with success [57–59]. GM-CSF has been shown to offer some protection against invasive aspergillosis; in one clinical trial in patients with acute myelogenous leukemia, decreasing the fungal infection-related mortality from 19–2% [60]. A small pilot study of GM-CSF in combination with AmB for treatment of proven fungal infection included two patients with refractory aspergillosis, with one showing a partial response and the other failing therapy [61]. A neutropenic rabbit model demonstrated that prophylactic administration of M-CSF 3 days prior to inoculation and then throughout neutropenia augmented pulmonary host defenses against invasive aspergillosis, leading to increased survival and greater numbers of activated pulmonary alveolar macrophages compared to controls [62]. A phase I trial of M-CSF in patients suggested some benefit in patients with *Aspergillus* infections, but an insufficient number of patients were treated to show a statistical benefit [56,63]. However, the use of GM-CSF is not without its concerns, as bone marrow recovery may lead to liquefaction of pulmonary foci and to potential erosive bleeding caused by an increased inflammatory response, especially in the first week following cavitation [64,65]. Excessive TNF toxicities in doses required to have a biologically useful effect preclude safe administration in humans [66,67]. There are case reports of the successful use of antifungals with IFNγ for treatment in CGD patients [68,69]. GM-CSF treatment of neutrophils with voriconazole increases activity against hyphae compared to control neutrophils and voriconazole, but no comparable effect was seen on monocytes [49,70]. An *in vivo* additive effect was also found with G-CSF and posaconazole in one study [71].

Conclusion

Thus, improved definition of the Th1/Th2 balance is essential for future prospects for immunotherapy, antimicrobial chemotherapy, and vaccination.

References

1 Hogaboam CM, Carpenter KJ, Schuh JM, Buckland KF. *Aspergillus* and asthma – any link? *Med Mycol* 2005; **43**(Suppl.1): S197–S202.

2 Moss RB. Pathophysiology and immunology of allergic bronchopulmonary aspergillosis. *Med Mycol* 2005; **43**(Suppl.1): S203–S206.

3 Stevens DA, Moss RB, Kurup VP, *et al.* and the Cystic Fibrosis Foundation Consensus Conference. Allergic broncho-pulmonary aspergillosis in cystic fibrosis, state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003; **37**(Suppl. 3): S225–S264.

4 Schaffner A, Douglas H, Braude A. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus*. Observations on these two lines of defense *in vivo* and *in vitro* with human and mouse phagocytes. *J Clin Invest* 1982; **69**: 617–631.

5 Roilides E, Blake C, Holmes A, Pizzo PA, Walsh TJ. Granulocyte-macrophage colony-stimulating factor and interferon-gamma prevent dexamethasone-induced immunosuppression of antifungal monocyte activity against *Aspergillus fumigatus* hyphae. *J Med Vet Mycol* 1996; **34**: 63–69.

6 Brummer E, Kamberi M, Stevens DA. Regulation by granulocyte-macrophage colony-stimulating factor and/or steroids given *in vivo* of proinflammatory cytokine and chemokine production by bronchoalveolar macrophages in response to *Aspergillus fumigatus*. *J Infect Dis* 2003; **187**: 705–709.

7 Brummer E, Maqbool A, Stevens DA. *In vivo* GM-CSF prevents dexamethasone suppression of killing of *Aspergillus fumigatus* conidia by bronchoalveolar macrophages. *J Leukoc Biol* 2001; **70**: 868–872.

8 Brummer E, Maqbool A, Stevens DA. Protection of peritoneal macrophages by granulocyte/macrophage colony-stimulating factor (GM-CSF) against dexamethasone suppression of killing of *Aspergillus*, and the effect of human GM-CSF. *Microbes Infect* 2002; **4**: 133–138.

9 Brummer E, Maqbool A, Stevens DA. Protection of bronchoalveolar macrophages by granulocyte-macrophage colony-stimulating factor against dexamethasone suppression of fungicidal activity for *Aspergillus fumigatus* conidia. *Med Mycol* 2001; **39**: 509–515.

10 Kamberi M, Brummer E, Stevens DA. Regulation of bronchoalveolar macrophage proinflammatory cytokine production by dexamethasone and granulocyte-macrophage colony-stimulating factor after stimulation by *Aspergillus* conidia and lipopolysaccharide. *Cytokine* 2002; **19**: 14–20.

11 Duong M, Ouellet N, Simard M, *et al*. Kinetic study of host defense and inflammatory response to *Aspergillus fumigatus* in steroid-induced immunosuppressed mice. *J Infect Dis* 1998; **178**: 1472–1482.

12 Mehrad B, Wiekowski M, Morrison BE, *et al*. Transient lung-specific expression of the chemokine KC improves outcome in invasive aspergillosis. *Am J Respir Crit Care Med* 2002; **166**: 1263–1268.
13 Morrison BE, Park SJ, Mooney JM, Mehrad B. Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis. *J Clin Invest* 2003; **112**: 1862–1870.
14 Mambula SS, San K, Henneke P, Golenbock DT, Levitz SM. Toll-like receptor (TLR) signaling in response to *Aspergillus fumigatus*. *J Biol Chem* 2002; **277**: 39320–39326.
15 Meier A, Kirschning CJ, Nikolaus T, *et al*. Toll-like receptor (TLR)2 and TLR4 are essential for *Aspergillus*-induced activation of murine macrophages. *Cell Microbiol* 2003; **5**: 561–570.
16 Netea MG, Warris A, Van der Meer JWM, *et al*. *Aspergillus fumigatus* evades immune recognition during germination through loss of Toll-like receptor-4-mediated signal transduction. *J Infect Dis* 2003; **188**: 320–326.
17 Bellocchio S, Montagnoli C, Bozza S, *et al*. The contribution of the Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens *in vivo*. *J Immunol* 2004; **172**: 3059–3069.
18 Bellocchio S, Moretti S, Perruccio K, *et al*. TLRs govern neutrophil activity in aspergillosis. *J Immunol* 2004; **173**: 7406–7415.
19 Bozza S, Gaziano R, Spreca A, *et al*. Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. *J Immunol* 2002; **168**: 1362–1371.
20 Romani L. Immunity to fungal infections. *Nature Rev Immunol* 2004; **4**: 1–13.
21 Netea MG, Van der Meer JWM, Sutmiller RP, Adema GJ, Kullberg B-J. From the Th1/Th2 paradigm towards a Toll-like receptor/T-helper bias. *Antimicrob Agents Chemother* 2005; **49**: 3991–3996.
22 Rivera A, Van Epps HL, Hohl TM, Rizzuto G, Pamer EG. Distinct $CD4^+$-T-cell responses to live and heat-inactivated *Aspergillus fumigatus* conidia. *Infect Immun* 2005; **73**: 7170–7179.
23 Montagnoli C, Fallarino F, Gaziano R, *et al*. Immunity and tolerance to *Aspergillus* involve functionally distinct regulatory T cells and tryptophan catabolism. *J Immunol* 2006; **176**: 1712–1723.
24 Cenci E, Perito S, Ensole K-H, *et al*. Th1 and Th2 cytokines in mice with invasive aspergillosis. *Infect Immun* 1997; **65**: 564–570.
25 Hebart H, Bollinger C, Fisch P, *et al*. Analysis of T-cell responses to *Aspergillus fumigatus* antigens in healthy individuals and patients with hematologic malignancies. *Blood* 2002; **100**: 4521–4528.
26 Bellocchio S, Bozza S, Montagnoli C, *et al*. Immunity to *Aspergillus fumigatus*: the basis for immunotherapy and vaccination. *Med Mycol* 2005; **43**(Suppl. 1): S181–S188.
27 Roilides E, Uhlig K, Venzon D, Pizzo PA, Walsh TJ. Prevention of corticosteroid-induced suppression of human polymorphonuclear leukocyte-induced damage of *Aspergillus fumigatus* hyphae by granulocyte colony-stimulating factor and interferon-γ. *Infect Immun* 1993; **61**: 4870–4877.
28 Nagai H, Guo J, Choi H, Kurup V. Interferon-γ and tumor necrosis factor-α protect mice from invasive aspergillosis. *J Infect Dis* 1995; **172**: 1554–1560.
29 Grazziutti ML, Rex JH, Cowart RE, *et al*. *Aspergillus fumigatus* conidia induce a Th1-type cytokine response. *J Infect Dis* 1997; **176**: 1579–1583.
30 Roilides E, Katsifu H, Walsh TJ. Pulmonary host defenses against *Aspergillus fumigatus*. *Res Immunol* 1998; **149**: 454–465.
31 Schelenz S, Smith DA, Bancroft GJ. Cytokine and chemokine responses following pulmonary challenge with *Aspergillus fumigatus*: obligatory role of TNF-α and GM-CSF in neutrophil recruitment. *Med Mycol* 1999; **37**: 183–194.
32 Mehrad B, Striefer RM, Standiford TJ. Role of TNF-α in pulmomary host defense in murine invasive aspergillosis. *J Immunol* 1999; **162**: 1633–1640.
33 Brieland JK, Jackson C, Menzel F, *et al*. Cytokine networking in lungs of immunocompetent mice in response to inhaled *Aspergillus fumigatus*. *Infect Immun* 2001; **69**: 1554–1560.
34 Phadke AP, Mehrad B. Cytokines in host defense against *Aspergillus*: recent advances. *Med Mycol* 2005; **43**(Suppl. 1): S173–S176.
35 Warris A, Netea MG, Verweij PE, *et al*. Cytokine responses and regulation of interferon-gamma release by human mononuclear cells to *Aspergillus fumigatus* and other filamentous fungi. *Med Mycol* 2005; **43**: 613–621.
36 Cenci E, Mencacci A, Del Sero G, *et al*. Interleukin-4 causes susceptibility to invasive pulmonary aspergillosis through suppression of protective type 1 responses. *J Infect Dis* 1999; **180**: 1957–1968.
37 Taramelli D, Malabarba MG, Sala G, Basilico N, Cocuzza G. Production of cytokines by alveolar and peritoneal macrophages stimulated by *Aspergillus fumigatus* conidia or hyphae. *J Med Vet Mycol* 1996; **34**: 49–56.
38 Bozza S, Peruccio K, Montagnoli C, *et al*. A dendritic cell vaccine against invasive aspergillosis in allogeneic hematopoietic transplantation. *Blood* 2003; **102**: 3807–3814.
39 Williams DM, Wiener MH, Drutz DJ. Immunologic studies of disseminated infection with *Aspergillus fumigatus* in the nude mouse. *J Infect Dis* 1981; **143**: 726–733.
40 Cenci E, Mencacci A, Fe d'Ostiani C, *et al*. Cytokine- and T helper-dependent lung mucosal immunity in mice with invasive pulmonary aspergillosis. *J Infect Dis* 1998; **178**: 1750–1760.
41 Cenci E, Mencacci A, Fe d'Ostiani C, *et al*. Cytokine- and T-helper dependent immunity in murine aspergillosis. *Res Immunol* 1998; **149**: 445–454.
42 Khatami S, Brummer E, Stevens DA. Effects of granulocyte-macrophage colony stimulating factor (GM-CSF) *in vivo* on cytokine production and proliferation by spleen cells. *Clin Exp Immunol* 2001; **125**: 198–201.
43 Choi J-H, Brummer E, Kang YJ, Jones PP, Stevens DA. IkB and NFkB in GM-CSF antagonism of dexamethasone suppression of macrophage response to *Aspergillus fumigatus* conidia. *J Infect Dis* 2006; **193**: 1023–1028.
44 Clemons KV, Calich VLG, Burger E, *et al*. Pathogenesis I: interaction of host cells and fungi. *Med Mycol* 2000; **38**(Suppl. 1): 99–111.
45 Clemons KV, Grunig G, Sobel RA, *et al*. Role of IL-10 in invasive aspergillosis: increased resistance of IL-10 gene knockout mice to lethal systemic aspergillosis. *Clin Exp Immunol* 2000; **122**: 186–191.
46 Roilides E, Sein T, Roden M, Schaufele RL, Walsh TJ. Elevated serum concentrations of IL-10 in non-neutropenic patients with invasive aspergillosis. *J Infect Dis* 2001; **183**: 518–520.
47 Balloy V, Huerre M, Latgé J-P, Chignard M. Differences in patterns of infection and inflammation for corticosteroid treatment and chemotherapy in experimental invasive pulmonary aspergillosis. *Infect Immun* 2005; **73**: 494–503.
48 Stevens DA. Combination immunotherapy and antifungal chemotherapy. *Clin Infect Dis* 1998; **26**: 1266–1269.

49 Stevens DA, Kullberg BJ, Brummer E, *et al*. Combined treatment: antifungal drugs with antibodies, cytokines or drugs. *Med Mycol* 2000; **38**(Suppl. 1): 305–315.

50 Saxena S, Bhatnagar PK, Ghosh PC, Usha Sharma P. Effect of amphotericin B lipid formulation on immune response in aspergillosis. *Int J Pharmaceut* 1999; **188**: 19–30.

51 Bellocchio S, Gaziano R, Bozza S, *et al*. Liposomal amphotericin B activates antifungal resistance with reduced toxicity by diverting Toll-like receptor signalling from TLR-2 to TLR-4. *J Antimicrob Chemother* 2005; **55**: 214–222.

52 Polak-Wyss A. Protective effect of human granulocyte colony-stimulating factor (hGCSF) on *Cryptococcus* and *Aspergillus* infections in normal and immunosuppressed mice. *Mycoses* 1991; **34**: 205–215.

53 Liles WC, Huang JE, van Burik JH, Bowden RA, Dale DC. Granulocyte colony-stimulating factor administered *in vivo* augments neutrophil-mediated activity against opportunistic fungal pathogens. *J Infect Dis* 1997; **175**: 1012–1015.

54 Geller RB. Use of cytokines in the treatment of acute myelocytic leukemia: a critical review. *J Clin Oncol* 1996; **14**: 1371–1382.

55 Roilides E, Holmes A, Blake C, Venzon D, Pizzo PA, Walsh TJ. Antifungal activity of elutriated human monocytes against *Aspergillus fumigatus* hyphae: enhancement by granulocyte-macrophage colony stimulating factor and interferon-gamma. *J Infect Dis* 1994; **170**: 894–899.

56 Nemunaitis J. Use of macrophage colony-stimulating factor in the treatment of fungal infections. *Clin Infect Dis* 1998; **26**: 1279–1281.

57 Abu Jawdeh L, Haidar R, Bitar F, *et al*. *Aspergillus* vertebral osteomyelitis in a child with a primary monocyte killing defect: response to GM-CSF therapy. *J Infect* 2000; **41**: 97–100.

58 Bodey GP. The potential role of granulocyte-macrophage colony stimulating factor in therapy of fungal infections: a commentary. *Eur J Clin Microbiol Infect Dis* 1994; **13**: 363–366.

59 Boots RJ, Paterson DL, Allworth AM, Faoagali JL. Successful treatment of postinfluenza pseudomembranous necrotising bronchial aspergillosis with liposomal amphotericin, inhaled amphotericin B, gamma interferon and GM-CSF. *Thorax* 1999; **54**: 1047–1049.

60 Rowe JM, Andersen JW, Maza J, *et al*. A randomized, placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (>55 to 70 years of age) with acute myelogenous leukemia: a study of the Eastern Cooperative Oncology Group (E1490). *Blood* 1995; **86**: 457–462.

61 Bodey GP, Anaissie E, Gutterman J, Vadhan-Raj S. Role of granulocyte macrophage colony-stimulating factor as adjuvant therapy for fungal infections in patients with cancer. *Clin Infect Dis* 1993; **17**: 705–707.

62 Gonzalez CE, Lyman CA, Lee S, *et al*. Recombinant human macrophage colony-stimulating factor augments pulmonary host defenses against *Aspergillus fumigatus*. *Cytokine* 2001; **15**: 87–95.

63 Nemunaitis J, Meyers JD, Buckner CD, *et al*. Phase I trial of recombinant human macrophage colony-stimulating factor (rhM-CSF) in patients with invasive fungal infections. *Blood* 1991; **78**: 907–913.

64 Albelda SM, Talbot GH, Gerson ST, *et al*. Pulmonary cavitation and massive hemoptysis in invasive pulmonary aspergillosis. Influence of bone marrow recovery in patients with acute leukemia. *Am Rev Respir Dis* 1985; **131**: 115–120.

65 Groll A, Renz S, Gerein V, Schwabe D, Katschan G, Schneider M, Hubner K, Kornhuber B. Fatal haemoptysis associated with invasive pulmonary aspergillosis treated with high-dose amphotericin B and granulocyte–macrophage colony-stimulating factor (GM-CSF). *Mycoses* 1992; **35**: 67–75.

66 Roilides E, Dimitriadou-Georgiadu A, Sein T, Kadiltsoglou I, Walsh TJ. Tumor necrosis factor alpha enhances antifungal activities of polymorphonuclear and mononuclear phagocytes against *Aspergillus fumigatus*. *Infect Immun* 1998; **66**: 5999–6003.

67 Rodriguez-Adrian LJ, Grazziutti ML, Rex JH, Anaissie EJ. The potential role of cytokine therapy for fungal infections in patients with cancer: is recovery from neutropenia all that is needed? *Clin Infect Dis* 1998; **26**: 1270–1278.

68 Saulsbury FT. Successful treatment of *Aspergillus* brain abscess with itraconazole and interferon-gamma in a patient with chronic granulomatous disease. *Clin Infect Dis* 2001; **32**: 137–139.

69 Bernhisel-Broadbent J, Camargo EE, Jaffe HS, Lederman HM. Recombinant human interferon-gamma as adjunct therapy for *Aspergillus* infection in a patient with chronic granulomatous disease. *J Infect Dis* 1991; **163**: 908–911.

70 Vora S, Chauhan S, Brummer E, Stevens DA. Activity of voriconazole combined with neutrophils or monocytes against *Aspergillus fumigatus*: Effects of granulocyte colony stimulating factor and granulocyte-macrophage colony-stimulating factor. *Antimicrob Agents Chemother* 1998; **42**: 2299–2303.

71 Graybill JR, Bocanegra R, Najvar LK, Loebenberg D, Luther MF. Granulocyte colony-stimulating factor and azole antifungal therapy in murine aspergillosis: role of immune suppression. *Antimicrob Agents Chemother* 1998; **42**: 2467–2473.