Role of TNF-α in Pulmonary Host Defense in Murine Invasive Aspergillosis

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Invasive pulmonary aspergillosis is a feared complication of immunodeficiency. Its incidence has increased dramatically over the past 2 decades, in tandem with an increase in the number of immunocompromised hosts (1, 2). Invasive pulmonary aspergillosis, the commonest form of the disease (3), carries a crude mortality rate of >80% with current therapy (4). *Aspergillus* species are the second commonest fungal pathogen in immunocompromised hosts, and *A. fumigatus* is responsible for 90% of cases of invasive aspergillosis.

The respiratory tract is the portal of entry in the majority of cases of human invasive aspergillosis (3). It is postulated that innate immunity is the principal pathway by which *A. fumigatus* is cleared from the lung. This innate response consists of alveolar macrophages, which represent the first line of defense against conidia entering the alveoli, and recruited neutrophils. Neutrophils are believed to kill conidia that have survived to form hyphae and cause invasive infection (5). Various cells of macrophage lineage can engulf and kill *Aspergillus* conidia in vitro (6–8) and in vivo (9, 10). Both macrophages (6, 11) and polymorphonuclear cells (5, 12) have been shown to damage hyphae in vitro. When exposed to conidia in vitro, cells of macrophage lineage secrete inflammatory mediators, including the proximal cytokines TNF-α and IL-1 (13, 14), as well as cytokines that promote Th-1 phenotype immune responses (15).

TNF-α is a 17-kDa cytokine secreted predominantly by various macrophage populations, including alveolar macrophages. It has shown to be a critical proximal signal in the initiation and maintenance of innate pulmonary immunity in animal models of pneumonia (16, 17) and human pneumonia (18). In the single study examining the role of TNF-α in invasive aspergillosis in vivo, immunocompetent animals administered *A. fumigatus* conidia i.v. had improved survival when treated with systemic murine rTNF-α (19). The role of TNF-α in pulmonary host defense against *A. fumigatus* has not been defined.

Given that TNF-α has been shown to be a critical mediator of innate immunity against several respiratory pathogens, attempts have been made to administer TNF-α to augment innate and acquired host responses. However, the therapeutic administration of TNF-α is limited by substantial dose-related toxicity, particularly when this cytokine is administered systemically. A TNF-α agonist peptide composed of the 11 amino acids that constitute the binding site of native human TNF-α to its receptors (referred to as TNF<sub>70–80</sub>)3 has recently been characterized (20, 21). Binding of TNF<sub>70–80</sub> to TNF-α receptors (both p55 and p75) has been shown to mediate many leukocyte-activating effects of native TNF-α, including stimulation of neutrophils for enhanced protease release and respiratory burst, and enhancement of neutrophil phagocytic activity and killing. When administered systemically, TNF<sub>70–80</sub> was associated with less toxicity than that observed with native TNF-α, due in part to the fact that this peptide did not alter adhesive properties of the endothelium (20). This peptide has been successfully administered directly

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3 Abbreviations used in this paper: TNF<sub>70–80</sub>, TNF-α agonist peptide; MIP-1α, macrophage inflammatory protein-1α; MIP-2, macrophage inflammatory protein-2; i.v., intravenous; MPO, myeloperoxidase; CCR-1, C-C chemokine receptor-1.
into the lungs of mice with Gram-negative bacterial pneumonia, although the beneficial effects of TNF-α, MIP-1α, MIP-2, and KC were observed only when the peptide was administered 3 or 7 days before, but not concomitant with, bacterial challenge (22).

In the current study we hypothesized that TNF-α is an important proximal signal in murine invasive pulmonary aspergillosis. We tested this hypothesis by assessing the production of TNF-α in the lungs of normal and immunocompromised mice challenged with A. fumigatus conidia. We next examined the effect of TNF-α depletion on survival, the burden of fungal organisms, and the host inflammatory response. Finally, the effects of intrapulmonary delivery of native TNF-α or TNF-70–80 on survival in cyclophosphamide-treated animals were determined.

Materials and Methods

Reagents

Polyclonal anti-murine TNF-α, MIP-1α, JE, MIP-2, and KC Abs used in the ELISAs were produced by immunization of rabbits with murine recombinant cytokines in multiple intradermal sites with CFA. Carrier-free recombinant murine rTNF, MIP-1α, MIP-2, and KC were purchased from R&D Systems (Minneapolis, MN). To TNF-α neutralization experiments, 0.5 ml of rabbit anti-murine TNF-α serum or control rabbit serum was administered i.p. 1 day before A. fumigatus administration. The i.p. injections of 0.25 ml of antisera or control rabbit serum were repeated every 4 h for two additional doses. TNF-70–80 and control peptides were synthesized at the University of Michigan Protein and Carbohydrate Structure Facility and were composed of the amino acid sequences H-Pro-Ser-Thr-His-Val-Leu-Ileu-Thr-His-Thr-Ieu-OH and H-Gly-Gly-Asp-Pro-Gly-Ileu-Val-Thr-His-Ser-OH, respectively (20). Both peptides were purified by HPLC and mass spectrometry, and contained no detectable LPS, as determined by Limulus lyase assay (ICN Biomedicals, Costa Mesa, CA).

Animals

Specific pathogen-free BALB/c mice (8-week-old females; Charles River Breeding Laboratories, Wilmington, MA) were used in all experiments. All mice were housed in specific pathogen-free conditions within the animal care facility at the University of Michigan until the day of sacrifice. Immunocompromised animals were treated with 200 mg/kg of cyclophosphamide (Sigma, St. Louis, MO), administered i.p., 4 days before intra-tracheal (i.t.) inoculation. Treatment with cyclophosphamide resulted in peripheral neutropenia (absolute neutrophil count, <200 cells/µl) by days 6 and 7 after treatment, with a return of peripheral counts to pretreatment levels by day 10.

Preparation and administration of A. fumigatus conidia

We chose to use A. fumigatus strain 13073 (American Type Culture Collection, Manassas, VA) in our studies, as this strain has previously been shown to induce invasive aspergillosis in immunocompromised mice (23). The organism was grown on Sabouraud dextrose agar plates (Becton Dickinson, Cockeysville, MD) for 7–10 days at 37°C. The surface of each plate was then washed with 100 ml of sterile 0.1% Tween-80 (SigmaUltra, St. Louis, MO) in normal saline. The resulting suspension of conidia was filtered through sterile gauze to remove clumps and hyphal debris, and then washed once and resuspended in 4 ml of 0.1% Tween-80. The concentration of A. fumigatus conidia in the suspension was determined by a particle counter (22 particle analyzer, Coulter, Hialeah, FL). The suspension was then diluted to the desired concentration, and the concentration was again measured before administration. In preliminary experiments, the number of particles determined by the particle counter was in close agreement with the number of viable colony-forming units found by serial dilution and plating of the suspension. On the day of inoculation, each animal was anesthetized with 1.8–2 mg of pentobarbital i.p. Using standard aseptic technique, the trachea was exposed and a 30-µl inoculum (A. fumigatus suspension or 0.1% Tween-80) was administered via a sterile 26-gauge needle. The skin incision was closed with surgical staples. In all experiments, immunocompetent animals were challenged with 1–2 × 106 conidia, and cyclophosphamide-treated animals were challenged with 1–2 × 105 conidia.

Lung harvest

At designated time points, the mice were sacrificed by CO2 asphyxiation. The chest cavity was opened aseptically, and the pulmonary vasculature was perfused with PBS via the right ventricle. For histologic examination, lungs were perfused with 1 ml of 4% paraformaldehyde in PBS, inflated with 1 ml of 4% paraformaldehyde in PBS via the trachea, and then excised en bloc. Lungs for various assays were perfused with 1 ml of PBS containing 5 mM EDTA, removed, frozen in liquid nitrogen, and stored at −20°C until the day of the assay. Lungs for cytokine and myeloperoxidase (MPO) assays were homogenized in 1.8–2 ml of pentobarbital (1 mg/ml) in 1.2% compound 450 inhibitor mixture buffer (Boehringer Mannheim, Mannheim, Germany) in PBS using a tissue homogenizer (Biospec Products, Bartlesville, OK). A 900-µl aliquot of PBS was added to 900 µl from each sample, sonicated for 10 s, and centrifuged at 500 × g for 10 min. Supernatants were passed through a 0.45-µm pore size filter (Gelman, Ann Arbor, MI) and stored at 4°C for cytokine ELISA.

Lung chitin assay

Given that molds (including the Aspergillus species) do not reliably form reproductive units in tissue, we employed an assay for chitin to measure the burden of organisms in lungs. Chitin, a component of the hyphal wall, is absent from mammalian cells and conidia. The assay was adapted from a previously described method (24). Lungs were homogenized in 5 ml of distilled water and centrifuged (1500 × g, 5 min, 20°C). The supernatants were discarded, and pellets were resuspended in sodium lauryl sulfate (3%, w/v) and heated at 100°C for 15 min. Samples were then centrifuged (1500 × g, 5 min, 20°C), and pellets were washed with distilled water and resuspended in 3 ml of KOH (120%, w/v). Samples were heated at 130°C for 60 min. After cooling, 8 ml of ice-cold ethanol (75%, v/v) was added to each sample, and tubes were shaken until ethanol and KOH had made one phase. Samples were incubated in ice for 15 min, and 0.3 ml of cell suspension (supernatant of 1 g of Celite 545 (Fisher Scientific, Pittsburgh, PA) added to 75% ethanol and allowed to stand for 2 min) was added to each. Samples were centrifuged (1500 × g, 5 min, 4°C), and supernatants were discarded. Pellets were washed once with ethanol (40%, v/v) and twice with distilled water, and resuspended in 0.5 ml of distilled water. Standards, consisting of 0.2 ml of distilled water and 0.2 ml of glucosamine (10 µg/ml) were made up. NaNO3 (0.2 ml; 5%, w/v), and KHSO3 (0.2 ml; 5%, w/v) were added to each standard, and NaNO3 (0.5 ml; 5%, w/v) and KHSO3 (0.5 ml; 5%, w/v) were added to each tissue prep; all samples were mixed gently for 15 min and then centrifuged (1500 × g, 2 min, 4°C). Two 0.6-ml aliquots of supernatant from each tissue prep were transferred to separate tubes. Ammonium hydroxide (0.2 ml) was added and all tubes were shaken vigorously for 5 min. A fresh solution of 3-methyl-2-thiazolone hydrazide HCl monohydrate (50 mg in 10 ml of distilled water) was made, and 0.2 ml was added to each tube. Samples were then heated to 100°C for 3 min and cooled. FeCl3·6H2O (0.2 ml; 0.83%, w/v) was added to each, and OD was measured at 650 nm after 25 min. Chitin content, measured in glucosamine equivalents, was measured by the following formula: chitin content = ([5 × (OD of organ−OD of control)])/(OD of glucosamine − OD of water).

Lung MPO activity

Lung MPO activity was measured as a marker of neutrophil sequestration, as described previously (25). Briefly, a 100-µl aliquot of each lung homogenate was added to 100 µl of a buffer containing 50 mM potassium phosphate (pH 6.0), 5% hexadecyltrimethylammonium bromide, and 5 mM EDTA. Samples were sonicated for 5 min, and centrifuged at 15000 × g for 10 min. The supernatant was mixed 1/15 with assay buffer and read at 490 nm. MPO units were calculated as the change in absorbance over time.

Cytokine ELISA

Murine TNF-α, MIP-1α, JE, MIP-2, and KC were quantified using a modification of a double ligand method, as described previously (26). Briefly, flat-bottom 96-well microtiter plates (Immunno-Plate I 96-F; Nunc, Nopenhagen, Denmark) were coated with 50 µl of well of rabbit anti-cytokine Ab (1 µg/ml in 0.6 M NaCl, 0.26 M H3BO3, and 0.08 NaOH, pH 9.6) for 16 h at 4°C and then washed with PBS (pH 7.5) and 0.05% Tween-20 (wash buffer). Microtiter plate nonspecific binding sites were blocked with 2% BSA in PBS and incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer, and unabsorbed and diluted (1/10) cell-free supernatants (50 µl) in duplicate were added to wells precoated with 50 µl of anti-cytokine Ab. Plates were washed four times, followed by the addition of 50 µl of well biotinylated rabbit anti-cytokine Abs (3.5 µg/ml in PBS (pH 7.5), 0.05% Tween-20, and 2% FCS), and plates were incubated for 30 min at 37°C. Plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA) was added, and plates were incubated for 30 min at 37°C. Plates were washed again four times, and chromogen substrate (Bio-Rad, Richmond, CA) was added. The plates were incubated at room temperature to the desired extinction, and the reaction was terminated.
with 50 μl/well of 3 M H₂SO₄ solution. Plates were read at 490 nm in an ELISA reader. Standards were 1/2 log dilutions of murine rTNF, MIP-1α, JE, MIP-2, or KC, from 1 pg/ml to 100 ng/ml. This ELISA method consistently detected the relevant cytokine at concentrations >25 pg/ml. The ELISAs did not cross-react with IL-1, IL-2, IL-4, IL-6, IL-10, or IFN-γ. In addition, each ELISA did not cross-react with other members of the murine chemokine family.

Statistical analysis

Data were analyzed by a Power Macintosh 8600/300 computer using the InStat version 2.01 statistical package (GraphPad, San Diego, CA). Survival data were compared using the Fisher’s exact test. All other data were expressed as the mean ± SEM and compared using unpaired two-tailed Mann-Whitney (nonparametric) test. The p values were considered statistically significant if they were <0.05.

Results

Development of pulmonary inflammation in normal and cyclophosphamide-treated mice after A. fumigatus administration

To characterize the host response to Aspergillus in immunocompetent and immunocompromised animals, normal and cyclophosphamide-treated BALB/c mice were challenged i.t. with 1–2 × 10⁷ and 1–2 × 10⁶ A. fumigatus conidia, respectively. Lungs were examined histologically at various time points after challenge. By 2 days after A. fumigatus administration in both normal and cyclophosphamide-treated mice, infiltration of inflammatory cells was noted within the alveolar and interstitial compartments in a patchy, peribronchial pattern (Fig. 1). The inflammatory cell infiltrate consisted predominantly of neutrophils and, to a lesser extent, mononuclear cells. By 4 days after A. fumigatus administration, cellular infiltrates were primarily mononuclear, with resolution occurring in immunocompetent mice, whereas a continued inflammatory response was noted in cyclophosphamide-treated animals up to 7 days after challenge (data not shown). Conidia were visible in normal mice 2 days after fungal inoculation and were nearly completely cleared by 5 days. In contrast, hyphae (the invasive form of A. fumigatus) were present only in cyclophosphamide-treated mice and were most numerous by 2–3 days after A. fumigatus administration. The inflammatory infiltrates observed corresponded to areas of conidial deposition in normal mice and to hyphae in cyclophosphamide-treated mice.

Lung TNF-α expression after administration of A. fumigatus conidia

We next correlated changes in lung TNF-α levels with the development of pulmonary inflammation in normal and cyclophosphamide-treated mice after administration of A. fumigatus conidia. As shown in Fig. 2A, a rapid increase in lung TNF-α levels was noted in immunocompetent mice when they were challenged with 1–2 × 10⁷ conidia, which reached a plateau by 24 h. Administration of 1–2 × 10⁶ A. fumigatus conidia to cyclophosphamide-treated mice resulted in a marked elevation in lung TNF-α levels by 1 day after administration, with maximal TNF-α levels at 2 days, and a return to baseline levels by 4 days after A. fumigatus administration (Fig. 2B). No significant induction of TNF-α was observed in the lungs of immunocompetent animals challenged with 1–2 × 10⁶ A. fumigatus conidia or in cyclophosphamide-treated animals challenged with 0.1% Tween-80 (vehicle) i.t.

Effect of TNF-α neutralization on survival in normal and immunocompromised mice inoculated with A. fumigatus conidia

Additional studies were undertaken to evaluate the contribution of TNF-α to survival after inoculation of conidia. To ascertain the
then inoculated with $1.0 \times 10^7$ conidia. Normal and cyclophosphamide-treated mice were inoculated with $9.3 \times 10^3$ conidia. A depicts lung TNF-$\alpha$ levels in immunocompetent mice after $A.\ fumigatus$ (1–2 $\times 10^9$ conidia) or vehicle challenge. B depicts lung TNF-$\alpha$ levels in cyclophosphamide-treated mice after $A.\ fumigatus$ challenge (1–2 $\times 10^9$ conidia), immunocompetent mice after $A.\ fumigatus$ challenge (1–2 $\times 10^9$ conidia), and cyclophosphamide-treated mice after vehicle challenge. Cy, cyclophosphamide. Experimental $n = 6$. *, $p < 0.05$ compared with animals receiving vehicle i.t.

To determine whether impaired fungal clearance in TNF-$\alpha$ depleted mice resulted in the appearance of both conidial and hyphal forms at 3 days after the administration of conidia, whereas only conidial forms of $A.\ fumigatus$ were seen in animals receiving preimmune serum (data not shown). In cyclophosphamide-treated animals, a marked increase in the number of hyphae was noted in animals treated with anti-TNF-$\alpha$ Ab, as evident on histology, compared with that in control animals (Fig. 4, B and D). To quantitate differences in fungal burden, lung chitin levels were determined in cyclophosphamide-treated animals. Lungs of TNF-depleted animals had a greater than twofold increase in chitin content compared with those of control animals (Fig. 5).

Effect of TNF-$\alpha$ neutralization on recruitment of inflammatory cells
To determine whether impaired fungal clearance in TNF-depleted animals occurred as a result of changes in lung neutrophil influx, lungs from cyclophosphamide-treated and normal mice given anti-TNF-$\alpha$ Ab or control serum were harvested for estimation of MPO activity, a surrogate measure of neutrophil presence. On the basis of studies showing the time of maximum expression of MPO in normal and cyclophosphamide-treated animals (not shown), lung
MPO levels were evaluated 1 day after inoculation in normal animals and 2 days after inoculation in cyclophosphamide-treated animals. In normal animals treated with anti-TNF, there was a 79% decrease in lung MPO levels compared with control values at 1 day, indicating reduced neutrophil recruitment in the absence of TNF. In cyclophosphamide-treated mice, the lung MPO level was reduced by 45% in TNF-depleted animals compared with that in controls at 2 days (Fig. 6).

Effect of TNF-α neutralization on expression of C-X-C and C-C chemokines

Given that TNF-α has been shown to be a potent inducer of both C-X-C and C-C chemokines, levels of the C-X-C chemokines MIP-2 and KC, and of the C-C chemokines, MIP-1α and JE, were measured in lung homogenates from normal and cyclophosphamide-treated mice. On the basis of preliminary studies showing the

FIGURE 4. Effect of TNF-α neutralization on lung histopathology after A. fumigatus challenge in cyclophosphamide-treated mice. Cyclophosphamide-treated animals were given rabbit anti-TNF-α serum or rabbit control serum 1 day before challenge with A. fumigatus. A and B depict representative lung hematoxylin and eosin (H&E) and Gomori methanamine silver (GMS) stains in cyclophosphamide-treated mice given control serum 3 days after inoculation of 1–2 × 10^7 A. fumigatus conidia (magnification, ×100). C and D depict representative lung H&E and GMS stains in cyclophosphamide-treated mice given anti-TNF-α serum 3 days after inoculation of 1–2 × 10^7 A. fumigatus conidia (magnification, ×100). Greater number of branching hyphae are visible in animals treated with anti-TNF. The data shown are representative of three experiments.

FIGURE 5. Effect of TNF-α neutralization on lung chitin after A. fumigatus challenge in cyclophosphamide-treated mice. Lung chitin levels in cyclophosphamide-treated mice given rabbit anti-murine TNF-α serum or control serum were determined 3 days after A. fumigatus challenge (1–2 × 10^7 conidia). Asp, A. fumigatus. Experimental n = 6. *, p < 0.05 compared with animals receiving control rabbit serum.

FIGURE 6. Effect of TNF-α neutralization on lung MPO activity after A. fumigatus challenge in immunocompetent and cyclophosphamide-treated mice. Lung MPO activity was measured 1 day after A. fumigatus challenge in normal mice (1–2 × 10^7 conidia) and 2 days after A. fumigatus challenge in cyclophosphamide-treated mice (1–2 × 10^6 conidia). Asp, A. fumigatus; Cy, cyclophosphamide. Experimental n = 6. *, p < 0.05 compared with animals receiving control rabbit serum.
time of maximum expression of these chemokines, levels were measured 1 day after *Aspergillus* inoculation in normal mice, and 3 days after *Aspergillus* inoculation in cyclophosphamide-treated mice. In both normal and cyclophosphamide-treated animals, there was a significant decrease in MIP-2 levels in anti-TNF-treated animals challenged with conidia compared with that in control animals receiving control rabbit serum. Treatment of normal or cyclophosphamide-treated animals with anti-TNF-α serum also resulted in significant reduction in the C-C chemokines MIP-1α and JE/monocyte chemoattractant protein-1 (Figs. 7 and 8).

**FIGURE 7.** Effect of TNF-α neutralization on lung MIP-2, KC, MIP-1α, and JE levels after *A. fumigatus* administration in immunocompetent mice. A and B depict C-X-C and C-C chemokine levels in immunocompetent mice 1 day after *A. fumigatus* challenge (1–2 × 10⁷), respectively. Experimental n = 6, *p < 0.05 compared with animals receiving control rabbit serum.

**FIGURE 8.** Effect of TNF-α neutralization on lung MIP-2, KC, MIP-1α, and JE levels after *A. fumigatus* administration in cyclophosphamide-treated mice. A and B depict C-X-C and C-C chemokine levels in cyclophosphamide-treated mice 3 days after *A. fumigatus* challenge (1–2 × 10⁶), respectively. Experimental n = 6, *p < 0.05 compared with animals receiving control rabbit serum.

**Discussion**

Invasive pulmonary aspergillosis is a common and devastating complication of immunosuppression. Given its poor outcome with current therapy, the precise mechanism of the immune response to *A. fumigatus* is of interest, since immunomodulation may be beneficial as adjunctive therapy.

We studied the pulmonary host response to *A. fumigatus* in normal and immunocompromised animal models. Despite the 10-fold greater concentration of conidia used in all experiments involving immunocompetent animals, normal hosts readily cleared these large inocula without developing invasive disease. Cyclophosphamide, an alkylating agent in common clinical use as a chemotherapeutic and immunosuppressive agent, was used to render animals susceptible to invasive disease. Cyclophosphamide induces a variety of effects on immunologically active cells; these include a dose-dependent depletion of circulating neutrophils as well as alterations in the number and function of circulating and pulmonary monocyte/macrophages and various lymphocyte populations (27, 28). Although peripheral blood neutropenia is an easily measurable
phosphamide-treated mice, TNF-α in humans. Therefore, this model has the advantage of notable pulmonary aspergillosis complicating severe immunosuppression pneumonia that clinically and histologically resembled invasive despite a reduced pool of available neutrophils. This is presumably due to pref-erent animals, the TNF-α response to the i.t. administration of A. fumigatus, which occurs before significant recruitment of other immune effector cells occurs (data not shown). This view is supported by the fact that TNF-α is released from alveolar macrophages coin-cubated with A. fumigatus conidia. Resident alveolar macrophages are most likely to be the source of this early and localized release of TNF, which occurs before significant recruitment of other immune effector cells occurs. Notably, neutrophils were present in the lungs of cyclophospha-mide-treated mice, TNF-α is produced in the lungs in re-sponse to the i.t. administration of A. fumigatus. In immunocompetent animals, the TNF-α level is significantly elevated by 8 h after A. fumigatus inoculation. Resident alveolar macrophages and neutrophils, and recruited monocyte/macrophages are most likely to be the source of the early and localized release of TNF, which occurs before significant recruitment of other immune effector cells occurs (data not shown). This view is supported by the fact that TNF-α is released from alveolar macrophages coin-cubated with A. fumigatus conidia in vitro. Elevated TNF-α at later time points probably represents release from various recruited cells, including neutrophils, and recruited monocyte/macrophages. Notably, neutrophils were present in the lungs of cyclophosphamide-treated mice infected with A. fumigatus while the animals had peripheral blood neutropenia. This is presumably due to prefer-ential accumulation of neutrophils at the site of inflammation despite a reduced pool of available neutrophils.

In vivo depletion of TNF-α resulted in increased fungal burden and mortality, which occurred in association with a reduction in lung neutrophil infiltration. TNF-α is likely to contribute to host defense against A. fumigatus by several mechanisms. Although not directly chemotactic for neutrophils, TNF-α induces leukocyte and endothelial cell expression of adhesion molecules, thus influencing neutrophil trafficking in the lungs. In addition, our studies indicate that the neutralization of TNF-α resulted in significant reductions in both C-X-C and especially C-C chemokines. Previous studies have suggested a role for chemokines in host defense against A. fumigatus: isolated rat alveolar macrophages produce MIP-1α, MIP-2, and KC, as well as TNF-α in response to A. fumigatus conidia in vitro, and knockout mice lacking CCR1, a receptor for MIP-1α and RANTES, develop disseminated infection when administered A. fumigatus i.v. (31). In this context, our study provides evidence that TNF-α drives the expression of both C-X-C and C-C chemokines, including MIP-1α, JE, and MIP-2, which may in part mediate the influx and activation of neutrophils and mononuclear cells in the lungs. Reduced amounts of MIP-2 and MIP-1α may explain the reduction in early neutrophil influx (and possibly activation), increased fungal burden, and increased mortality in TNF-depleted animals. In addition, MIP-1α and JE/ monocyte chemoattractant protein-1 may play important roles in mononuclear cell recruitment and activation in the setting of in-vasive pulmonary aspergillosis. Studies establishing causal rela-tionships are in progress.

The intratracheal administration of TNF-α provided survival benefits when given 3 days before the i.t. inoculation of A. fumigatus conidia, but not when given concomitantly with conidia. These results parallel our earlier observations in murine bacterial pneu-monia (22). The lack of a beneficial effect in animals treated with TNF-α concomitant with conidial administration may be due to several possible mechanisms. Firstly, TNF-α is more potent as a priming agent than as a direct activator of leukocyte respiratory burst and degranulation in vitro. Therefore, pretreatment with TNF-α may provide sufficient macrophage priming, whereas concomitant treatment may result in insufficient leukocyte activation. Alternatively, leukocyte release of proteolytic enzymes in response to infectious organisms within the airspace may lead to cytokine degradation and loss of biologic effects. Lastly, the administration of native TNF-α or TNF-α given at the time of fungal challenge may induce lung injury in the setting of pneu-monia, thereby negating any beneficial effect on mortality.

Given that immunocompromised patients at risk of developing pulmonary invasive aspergillosis are easily identifiable, early in-stitution of prophylactic immunomodulatory treatment is a poten-tial application of this study. Such clinical applications require further study.

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References
TNF-α IN MURINE INVASIVE ASPERGILLOSIS


