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Dectin-1 Fc Targeting of *Aspergillus fumigatus* Beta-Glucans Augments Innate Defense against Invasive Pulmonary Aspergillosis▽

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Invasive pulmonary aspergillosis (IPA) has significantly increased over the last decade. Here, a fusion protein consisting of the Dectin-1 extracellular domain linked to the Fc portion of murine immunoglobulin G1 augmented alveolar macrophage killing of *Aspergillus fumigatus* and shifted mortality associated with IPA via attenuation of *A. fumigatus* growth in the lung.

Immunosuppressed individuals undergoing solid organ or hematopoietic cell transplantation are at high risk for developing invasive fungal infections (3, 4). Among these, invasive pulmonary aspergillosis (IPA) caused by the fungal pathogen *Aspergillus fumigatus* is associated with an extraordinary mortality rate. A recent analysis of invasive fungal infections in patients with hematologic malignancies has reported an increase in infections caused by *A. fumigatus* from 0.9% to 2.9% between 1989 and 2003 (6).

We have previously reported that (i) interruption of *A. fumigatus* recognition by the beta-glucan receptor Dectin-1 attenuated alveolar macrophage (AM) inflammatory responses to *A. fumigatus* (11), (ii) beta-glucans were exposed at the highest levels in *A. fumigatus* swollen conidia (SC) (11), and (iii) a fusion protein consisting of the extracellular domain of Dectin-1 linked to the Fc portion of murine immunoglobulin G1 (Dectin-Fc) augmented innate killing of *Pneumocystis carinii* and attenuated the growth of *P. carinii* in the lungs of SCID mice (7). Although the role of antibody-mediated immunity in the host defense against *A. fumigatus* is poorly understood, it is recognized that antibodies may contribute to host cell effector functions (2). To this end, we hypothesize that Dectin-Fc will promote opsonic killing of *A. fumigatus* and augment its clearance from the lung during immunosuppression.

AMs were isolated from male C57BL/6 mice by bronchoalveolar lavage as previously described (10, 11). Animal studies were approved by the Children's Hospital of Pittsburgh Animal Research and Care Committee. AMs were cocultured with *A. fumigatus* (isolate 13073; ATCC) (5) SC (generated by incubation at 37°C for 6 h) (11) at a ratio of AM to conidia of 1:2 in the presence or absence of Dectin-Fc-conditioned supernatant (7). The development of Dectin-Fc and the adenoviral vector has been previously described (7). A viability control of *A. fumigatus* SC incubated with medium alone or Dectin-Fc was included. After 6 h at 37°C, total *A. fumigatus* RNA was isolated with the MasterPure yeast RNA kit (Epicenter, Madison, WI) (9) and reverse transcribed and the viability of *A. fumigatus* was quantified against a standard curve of diluted live *A. fumigatus* SC by real-time PCR measurement of the *A. fumigatus* 18S rRNA (GenBank accession no. AB008401) (1). As a validation of the real-time PCR method, heat-killed *A. fumigatus* SC did not yield a signal by real-time PCR and were unable to grow on potato dextrose agar plates (data not shown). Figure 1 shows that AMs were relatively ineffective at killing *A. fumigatus* SC after 6 h of coculture. However, addition of Dectin-Fc to the coculture dramatically enhanced killing by more than fourfold ($P < 0.001$; analyzed by the Student *t* test with GraphPad Prism version 5 statistical software). Thus, killing of *A. fumigatus* by AMs is enhanced when targeting *A. fumigatus* for opsonic elimination via beta-glucan recognition.

We next subjected mice to a level of immunosuppression that is permissive for the development of IPA (8, 9, 12). Four days and 1 day prior to *A. fumigatus* challenge, mice received 200 mg/kg and 150 mg/kg, respectively, of cyclophosphamide (Sigma) intraperitoneally. Mice further received 10 mg of cortisone (Sigma) subcutaneously 3 days prior to *A. fumigatus* challenge and again at the time of challenge. Forty-eight hours after immunosuppression was initiated, mice received adenoviral vectors encoding either Dectin-Fc (AdDectin-Fc) or firefly luciferase (AdLuc; control) intravenously. Forty-eight hours thereafter, mice (six per adenoviral group) were challenged intratracheally with 5×10^5, 5×10^4, or 1×10^4 *A. fumigatus* conidia. Four days after adenovirus administration, Dectin-Fc protein was detected at high levels in the lungs of immunosuppressed mice receiving AdDectin-Fc, but not AdLuc, as previously reported (7). The protective effects of Dectin-Fc at the higher two inoculum concentrations were significant but subtle and showed a shift in median survival time (MST) (5×10^5 conidia, 48 h versus 66 h for AdLuc and AdDectin-Fc, respectively; 5×10^4 conidia, 60 h versus 72 h for AdLuc and AdDectin-Fc, respectively; $P < 0.05$ for both inocula; survival analysis was performed with an asymmetrical 95% confidence interval and the Mantel-Cox log-rank test). Figure 2 shows that despite being challenged with a much lower dose of *A. fumigatus*, 1×10^4 conidia, the MST of AdLuc-treated mice was similarly 60 h. In contrast, mice that received AdDectin-Fc were significantly more protected from death and had an MST

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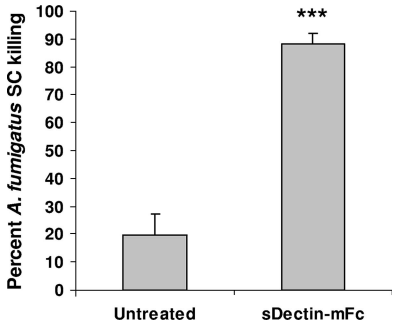

FIG. 1. Dectin-Fc enhances AM killing of *A. fumigatus* SC. AMs were isolated from 8- to 12-week-old male C57BL/6 mice and cocultured for 6 h with *A. fumigatus* SC at a ratio of macrophage to conidia of 1:2 in the presence or absence of Dectin-Fc (7). Controls included *A. fumigatus* cultured in medium alone or in the presence of Dectin-Fc. Thereafter, RNA was isolated from the contents of each well and a quantitative real-time PCR for *A. fumigatus* 18S rRNA was performed. Shown are cumulative results of three experiments. ***, $P < 0.001$. Data are expressed as mean percent killing plus the standard error of the mean.

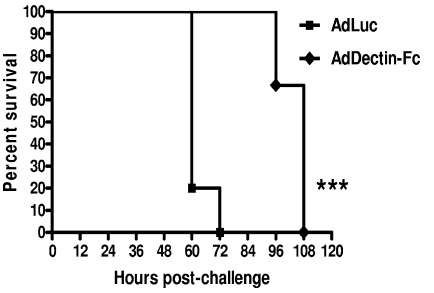

FIG. 2. Systemic administration of Dectin-Fc shifts mortality in an immunosuppressive model of IPA. Male 8- to 12-week-old C57BL/6 mice were immunosuppressed as described in the text and administered either AdDectin-Fc (7) or AdLuciferase (1×10^9 PFU intravenously in 100 μl). Forty-eight hours after adenoviral vector treatment, mice were intratracheally challenged with 1×10^4 *A. fumigatus* conidia in a volume of 50 μl and monitored for 5 days. Shown are results for six mice per adenoviral group. ***, $P < 0.001$. Data are expressed as percent survival.

of 108 h ($P < 0.001$). Thus, Dectin-Fc can preserve antifungal immunity in mice that were pharmacologically targeted to have severe suppression of innate immune responses.

Data presented in Fig. 2 indicated that Dectin-Fc shifted *A. fumigatus*-associated mortality in immunosuppressed mice. Immunosuppressed mice were therefore administered AdLuc and AdDectin-Fc as before and subsequently challenged with 1×10^4 conidia. At 24 and 48 h postinoculation, *A. fumigatus* lung burdens were analyzed by real-time PCR, a detection method reported to be superior to quantitative cultures or galactomannan enzyme immunoassay (9). To quantify the *A. fumigatus* lung burden, RNA was simultaneously isolated from 10-fold dilutions of live *A. fumigatus* conidia (beginning at 10^9), as well as the lung samples, with the MasterPure kit (9). Results showed that mice receiving either AdLuc or AdDectin-Fc had low levels of *A. fumigatus* organisms 24 h after receiving 1×10^4 conidia (AdLuc, $2.24 \times 10^2 \pm 1.39 \times 10^2$; AdDectin-Fc, $5.50 \times 10^2 \pm 2.90 \times 10^2$; data are expressed as the mean number of *A. fumigatus* 18S rRNA units per lung ± the standard error of the mean and are from one representative experiment of two, with five mice per group). However, *A. fumigatus* lung burdens in immunosuppressed mice receiving AdLuc dramatically increased by 48 h postinoculation ($1.41 \times 10^6 \pm 5.33 \times 10^5$). In contrast, mice receiving AdDectin-Fc had significantly lower *A. fumigatus* lung burdens by 48 h ($1.1 \times 10^4 \pm 6.7 \times 10^3$, $P < 0.05$; analyzed by the Student *t* test). Thus, the presence of Dectin-Fc in the lungs of immunosuppressed mice allows for better control of *A. fumigatus* overgrowth.

In conclusion, Dectin-Fc effectively targeted *A. fumigatus* via beta-glucan recognition and opsonic elimination without having to rely on the immune system to respond to a currently uncharacterized immunoprotective *A. fumigatus* antigen(s). Moreover, Dectin-Fc was effective during immunosuppression and therefore lays the foundation for Dectin-Fc prophylaxis for the treatment of IPA.

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We have no conflicts of interest to disclose.

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