Dectin-1 Fc targeting of *Aspergillus fumigatus* beta-glucans augments innate defense against invasive pulmonary aspergillosis

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Abstract

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 IgG1 augmented alveolar macrophage killing of *A. fumigatus* and shifted mortality associated with IPA via attenuation of *A. fumigatus* lung growth.
Immunosuppressed individuals undergoing solid organ or hematopoietic cell transplantation are at high risk for developing invasive fungal infections. 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 1989-2003.

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* (ii) beta-glucans were exposed at the highest levels in *A. fumigatus* swollen conidia (iii) a fusion protein consisting of the extracellular domain of Dectin-1 linked to the Fc portion of murine IgG1 (Dectin-Fc) augmented innate killing of *Pneumocystis carinii* and attenuated the growth of *P. carinii* in the lungs of SCID mice. Although the role of antibody-mediated immunity in host defense against *A. fumigatus* is poorly understood, it is recognized that antibodies may contribute to host cell effector functions. To this end, we hypothesize that Dectin-Fc will promote opsonic killing of *A. fumigatus* and augment lung clearance during immunosuppression.

AMs were isolated from male C57BL/6 mice by bronchoalveolar lavage as previously described. Animal studies were approved by the Children’s Hospital of Pittsburgh Animal Research and Care Committee. AMs were co-cultured with *A. fumigatus* (isolate 13073, ATCC) swollen conidia (SC; generated by incubation at 37°C for 6 h) at a AM to conidia ratio of 1:2 in the presence or absence of Dectin-Fc conditioned supernatant. The development of Dectin-Fc and the adenoviral vector has been previously described. A viability control of *A. fumigatus* SC incubated with media alone or Dectin-Fc was included. After 6 h at 37°C, total *A. fumigatus* RNA was isolated using the MasterPure™ yeast RNA kit (Epicentre, Madison, WI), reverse transcribed and the...
viability of \textit{A. fumigatus} quantified against a standard curve of diluted live \textit{A. fumigatus} SC using real-time PCR measurement of the \textit{A. fumigatus} 18S rRNA (GenBank number AB008401) \cite{1}. As a validation of the real-time PCR method, heat-killed \textit{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 \textit{A. fumigatus} SC after 6 h of co-culture. However, addition of Dectin-Fc to the co-culture dramatically enhanced killing by more than 4-fold (\( p < 0.001 \); analyzed with the Student's \( t \)-test using GraphPad Prism Version 5 statistical software). Thus, killing of \textit{A. fumigatus} by AMs is enhanced when targeting \textit{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 \cite{8,9,12}. Four days and 1 day prior to \textit{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 \textit{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 (\( n = 6 \) per adenoviral group) were challenged intratracheally with 5\( \times \)10\(^5\), 5\( \times \)10\(^4\) or 1\( \times \)10\(^4\) \textit{A. fumigatus} conidia. Four days after adenoviral administration, Dectin-Fc protein was detected at high levels in the lungs of immunosuppressed mice receiving AdDectin-Fc, but not AdLuc, as previously reported \cite{7}. The protective effects of Dectin-Fc in the higher two inocula were significant, but subtle, and showed a shift in median survival time (MST) (5\( \times \)10\(^5\) – 48 h vs. 66 h for AdLuc and AdDectin-Fc, respectively; 5\( \times \)10\(^4\) – 60 h vs. 72 h for AdLuc and AdDectin-Fc, respectively; \( p < 0.05 \) for both inocula; survival analysis performed using 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 \textit{A. fumigatus}, 1\( \times \)10\(^4\), the MST for AdLuc-treated mice was similarly 60 h. In contrast, mice that received AdDectin-Fc were significantly more protected from
mortality and had a MST of 108 h (p < 0.001). Thus, Dectin-Fc can preserve anti-fungal immunity in mice that were pharmacologically targeted to have severe suppression of innate immune responses.

Data presented in Figure 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 1x10⁴ conidia. At 24 and 48 h post-inoculation, *A. fumigatus* lung burden was analyzed by real-time PCR, a detection method reported to be superior than quantitative cultures or galactomannan enzyme immunoassay. To quantify the level of *A. fumigatus* lung burden, RNA was simultaneously isolated from 10-fold dilutions of live *A. fumigatus* conidia (beginning at 10⁰) as well as the lung samples using the MasterPure™ kit. Results showed that mice receiving either AdLuc or AdDectin-Fc had low levels of *A. fumigatus* organisms 24 h after receiving 1x10⁴ conidia (AdLuc: 2.24x10² ± 1.39x10², AdDectin-Fc: 5.50x10² ± 2.90x10²; data expressed as mean *A. fumigatus* 18S rRNA units per lung ± standard error of the mean from one representative experiment of two, n = 5 for each group). However, *A. fumigatus* lung burden in immunosuppressed mice receiving AdLuc dramatically increased by 48 h post-inoculation (1.41x10⁶ ± 5.33x10⁵). In contrast, mice receiving AdDectin-Fc had significantly lower *A. fumigatus* lung burden by 48 h (1.1x10⁴ ± 6.7x10³, p < 0.05; analyzed using the Student’s 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 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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References


Figure legends

**Figure 1. Dectin-Fc enhances alveolar macrophage killing of *A. fumigatus* swollen conidia.**
Alveolar macrophages were isolated from 8-12 week old, male C57BL/6 mice and co-cultured for 6 h with *A. fumigatus* SC at a macrophage to conidia ratio of 1:2 in the presence or absence of Dectin-Fc. 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 quantitative real-time PCR for *A. fumigatus* 18S rRNA was performed. The Figure illustrates cumulative results from three experiments. *** represents a p value of < 0.001. Data is expressed as mean percent killing ± SEM.

**Figure 2. Systemic administration of Dectin-Fc shifts mortality in an immunosuppressive model of IPA.** Male, 8-12 week old C57BL/6 mice were immunosuppressed as described in the text and administered either AdDectin-Fc or AdLuciferase (1x10⁹ PFU intravenously in 100 µl). Forty-eight hours after adenoviral vector treatment, mice were intratracheally challenged with 1x10⁴ *A. fumigatus* conidia in a volume of 50 µl and monitored for 5 days. The Figure illustrates results from 6 mice per adenoviral group. *** represents a p value of < 0.001. Data is expressed as percent survival.
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The figure shows a survival curve for two groups: AdLuc and AdDectin-Fc. The x-axis represents hours post-challenge, ranging from 0 to 120, and the y-axis represents percent survival, ranging from 0 to 100. The curve for AdLuc shows a significant decrease in survival compared to AdDectin-Fc, as indicated by the asterisks (***) above the curve.

The graph demonstrates the impact of the challenge on survival rates over time, with AdDectin-Fc maintaining a higher survival rate than AdLuc.