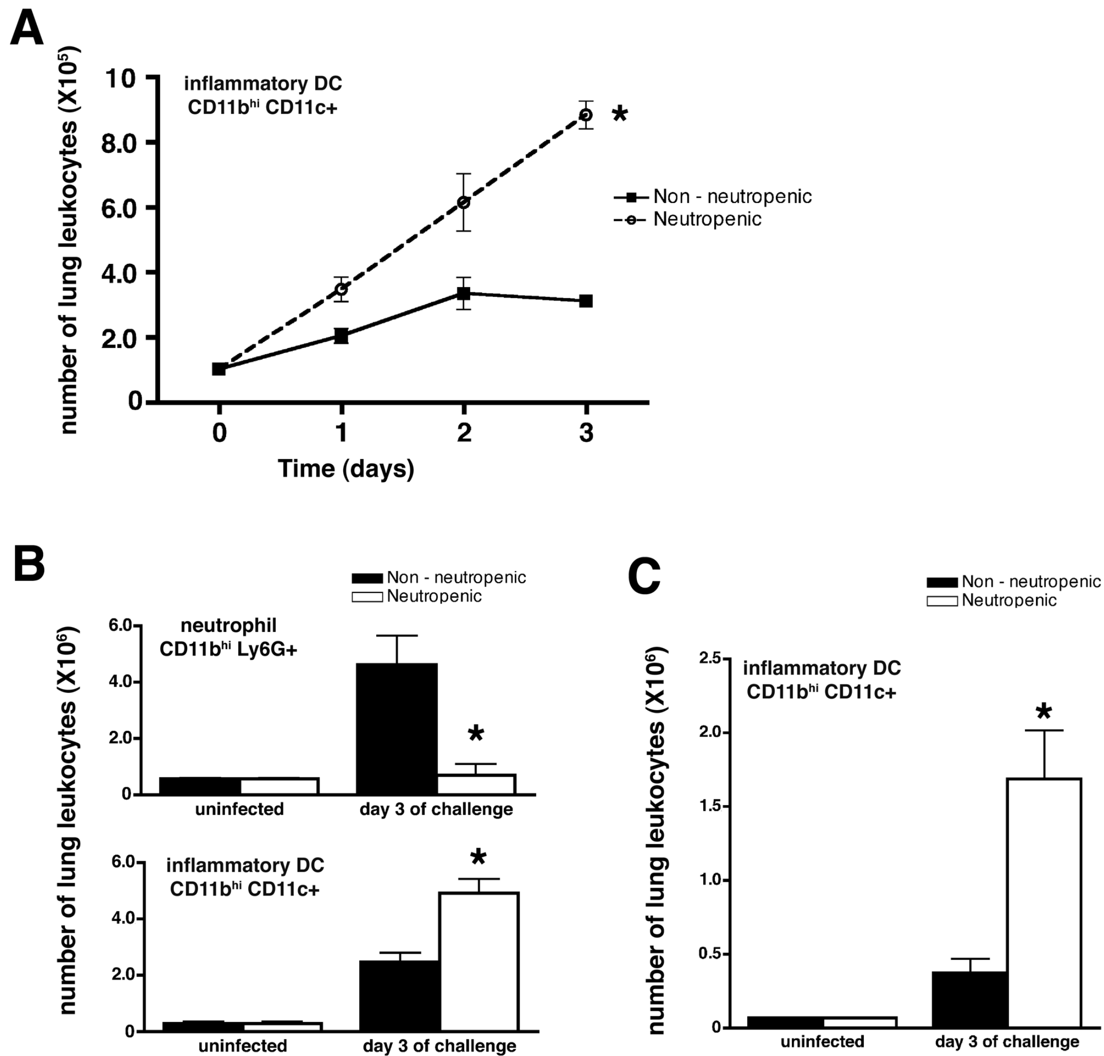
SUPPLEMENTAL DATA

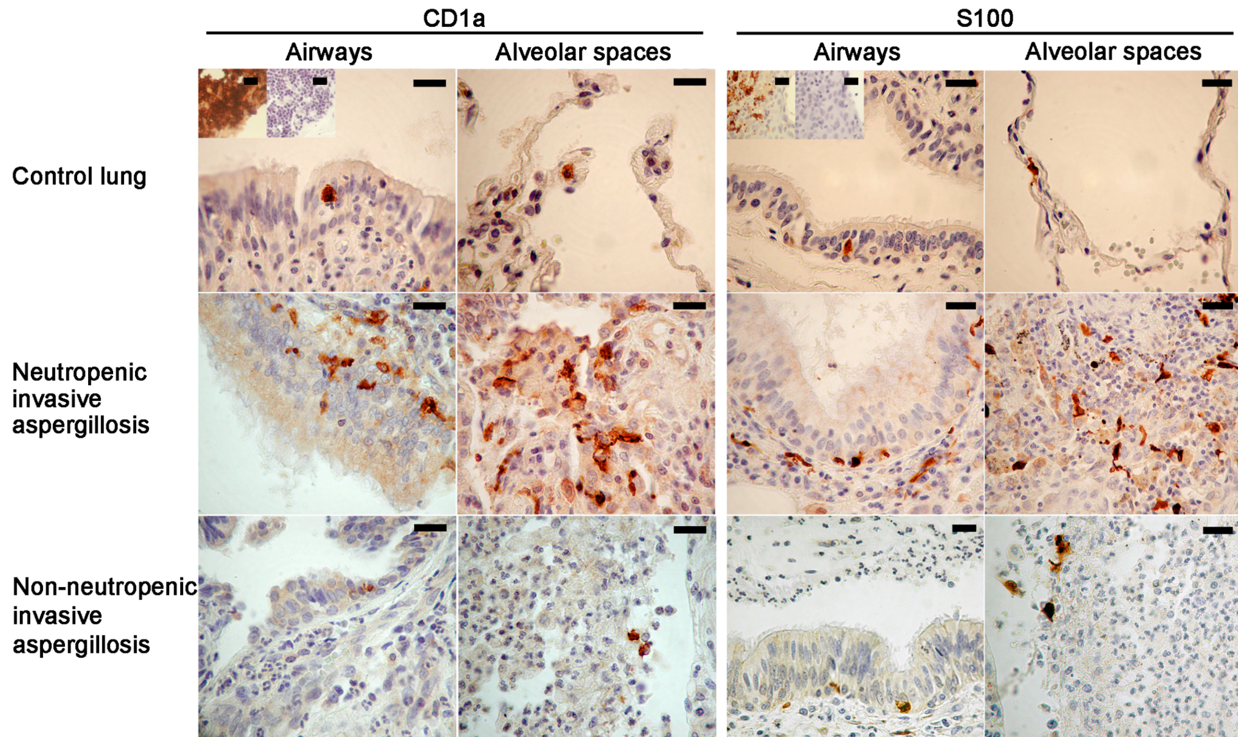
Neutropenia Enhances Lung Dendritic Cell Recruitment in Response to *Aspergillus* via a Cytokine-to-Chemokine Amplification Loop

Stacy J Park[1], Marie D Burdick[2], William K Brix[3], Mark H Stoler[3], David S Askew[4], Robert M Strieter[2], and Borna Mehrad[1,2,5]

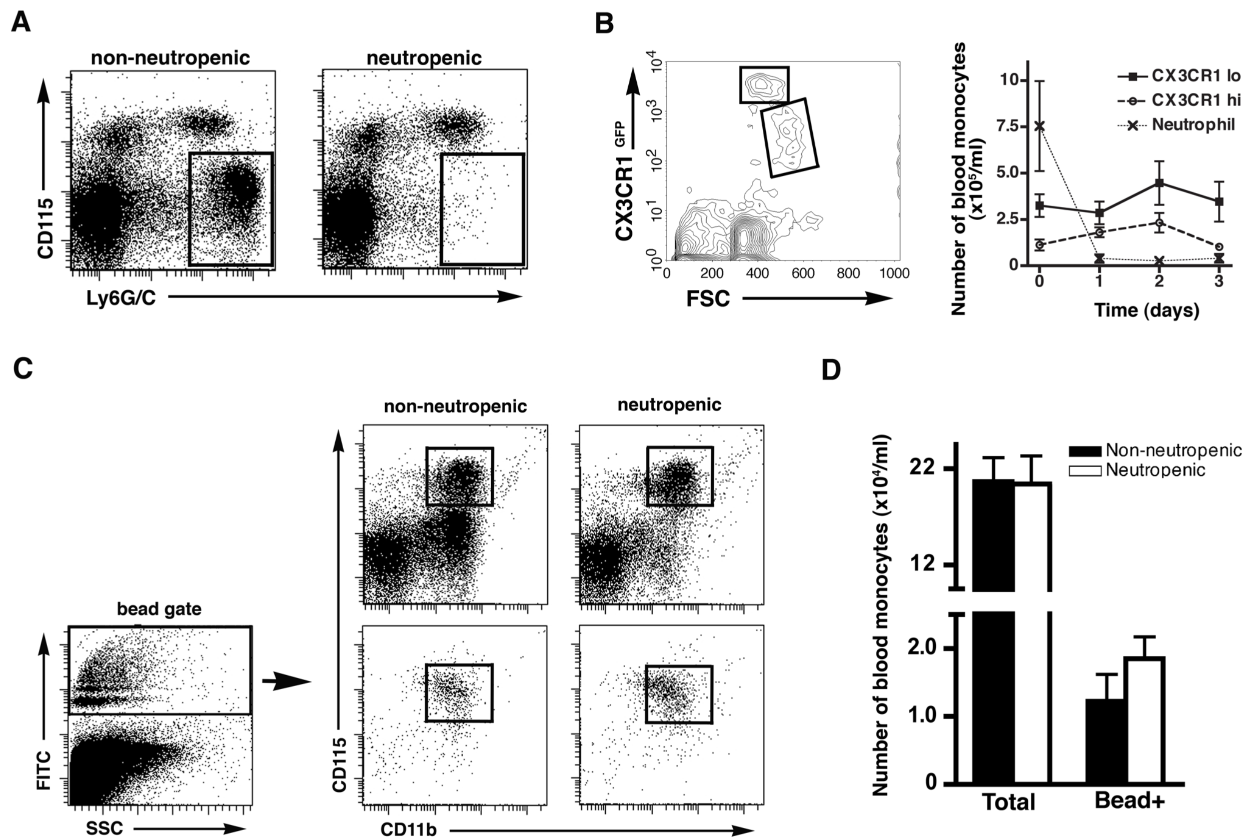
[1]Department of Microbiology, [2]Division of Pulmonary and Critical Care Medicine, Department of Medicine, [3]Department of Pathology and [5]Carter Center for Immunology, University of Virginia, Charlottesville, VA 22908; and [4]Department of Pathology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.



Supplemental figure 1. Effect of neutrophil depletion on the number of lung DCs in response to *Aspergillus*. **(A)** Number of lung inflammatory DC in mice with Ab-mediated neutrophil depletion (Gr-1, anti-Ly-6C/G, clone RB6-8C5) and mice treated with isotype control Ab were challenged with killed swollen conidia. **(B)** Number of lung neutrophils (top panel) and inflammatory DC (bottom panel) in neutropenic and non-neutropenic mice after challenge with killed *Aspergillus* hyphae, using an alternative neutrophil-depleting mAb (anti-Ly-6G, clone 1A8) as compared to animals treated with isotype control mAb. **(C)** Number of lung inflammatory DC after challenge with live hyphae from an attenuated mutant strain of *Aspergillus* strain of *A. fumigatus* in mice with Ab-mediated neutrophil depletion and mice treated with isotype control Ab. In all panels, data shown represent mean ± SEM; n = 4 for each group, representative data from 2 experiments. *, $p < 0.05$ comparing trend between the two groups over time.



Supplemental figure 2: Accumulation of lung DCs in neutropenic patients with invasive aspergillosis. Representative photomicrographs of the immunolocalization of DCs using CD1a and S100 in normal lung tissue (top panels), neutropenic invasive aspergillosis (middle panels) and non-neutropenic invasive aspergillosis (bottom panels). Insets show control thymic tissue labeled with Cd1a or S100 (left) or respective control Ab (right). Neutrophils are identifiable in lower panels based on their nuclear morphology. All scale bars are 20 µm; original magnifications were 400X for all the panels.



Supplemental figure 3. Blood monocyte analysis in neutropenic and non-neutropenic mice. **(A)** Representative flow cytometry plots in mice with or without administration of the Gr-1 mAb (anti-Ly-6G/C; clone RB6-8C5) on day 3 following challenge with killed *Aspergillus* hyphae, representative data from 2 experiments, *n* = 4 mice per group. **(B)** Representative flow cytometry plot and quantitative data in CX3CR1 $^{GFP/+}$ mice. Monocytes subsets and neutrophils were enumerated following administration of Gr-1 mAb on day -1 in uninfected mice. Data shown represent mean +/- SEM of *n* = 4 mice per group. **(C)** Representative flow cytometry plots and **(D)** quantitative data after labeling of circulating monocytes with FITC$^+$ beads in mice with Ab-mediated neutrophil depletion and mice treated with isotype control Ab on day 3 following challenge with killed *Aspergillus* hyphae. Top panel shows total monocytes based on CD115 and CD11b expression. Bottom panels show the same population gated on FITC$^+$ beads. Representative data from 2 experiments, *n* = 4 mice per group.