STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Title: Differences in pH-dependent cathepsin activity and ROS generation are unlikely to explain alveolar macrophage-mediated FVB innate resistance to *Pneumocystis*.

Background: *Pneumocystis* pneumonia (PCP), an AIDS defining illness, is caused by an opportunistic fungal pathogen that typically infects patients with depressed $CD4^+$ T cell numbers ($<200/mm^3$). Although most laboratory mouse strains including Balbc and C57BL/6, are susceptible to PC infection when depleted of $CD4^+$ T cells, the FVB strain has been shown to be resistant. FVB resistance requires the presence of alveolar macrophages, but the mechanism of resistance has not been determined.

Objective: To determine whether enhanced generation of antimicrobial ROS, or increased lysosomal protease activity in FVB macrophages is associated with resistance.

Methods: Alveolar macrophages isolated from the lungs of FVB, Balbc, and C57BL6 mice by bronchoalveolar lavage (BAL) were stimulated with live or heat-killed PC organisms. Reactive oxygen species (ROS) were measured using a plate-based luminol assay, while cathepsin activity was measured using MagicRed fluorescent substrate. In some experiments concentrated BAL fluid was added to the macrophage and PC cultures.

Results: CD4-depleted FVB mice were determined to be fully resistant to PC infection. However, when alveolar macrophages were depleted with clodronate liposomes, the resistant FVB mice became susceptible, suggesting that FVB AMs are directly responsible for resistance. To determine whether FVB AMs efficiently kill PC by producing more lysosomal proteases or toxic ROS than AMs from susceptible mice, in vitro co-cultures of AMs and PC were analyzed. There was no significant difference in lysosomal protease activity or ROS generation among the resistant and susceptible AMs following the interaction with PC, indicating that these factors are unlikely to play a role in resistance. Interestingly, most of the ROS produced in culture was found to be generated by the PC organisms themselves, and AMs appeared to produce little ROS after PC exposure. Heat-killed PC did not produce any ROS, and concentrated FVB BAL fluid nearly totally suppressed ROS generation by live PC.

Conclusion: Analysis of macrophage lysosomal protease activity and ROS generation following PC exposure suggests that these factors are not responsible for FVB resistance. In fact, all AMs tested unexpectedly produce little ROS when exposed to PC. In contrast, live PC produces ROS in culture, and FVB BAL fluid suppresses ROS production by PC through an as yet unknown mechanism.

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