Interactions of *Pneumocystis carinii* with Alveolar Macrophages

O'Bert RJ
Linke MJ
Walzer PD

VAMC Department of Internal Medicine, Division of Infectious Diseases

Introduction:
Pulmonary infection due to Pneumocystis carinii (Pc) is a frequent cause of mortality and morbidity in patients infected with the human deficiency virus (HIV) and other immunocompromised hosts. Once Pneumocystis reaches the alveolus, it encounters the alveolar lining layer, a thin film covering the epithelium containing pulmonary surfactant and inflammatory cells. Alveolar macrophages are the first line of defense and principal host effector cells against Pneumocystis. Interactions through the mannose and the Fc receptors have been shown to mediate binding, phagocytosis, and killing of Pneumocystis.

Rationale:
Conclusions based on previous studies of the interaction of Pneumocystis with alveolar macrophages have been limited due to study design and assay techniques. Our objective was to better analyze these interactions using a homologous source of reagents and a novel assay technique.

Methods:
Alveolar macrophages were isolated from Lewis rats by bronchoalveolar lavage and attached to 10-well printed glass microscope slides for 2h at 37°C at a concentration of 2x104 macrophages per well. Pneumocystis carinii organisms were isolated from infected rats and fluorescently labeled with Bodipy. These organisms were then incubated with the alveolar macrophages for 15, 30, 60 and 120 minutes at a concentration of 2x106 organisms per well. A second set of macrophages was incubated with labeled organisms for 2h at 4°C. Following the 4°C incubation, unattached organisms were removed and the slides incubated at 37°C for 0.5, 1 and 4 hours. Slides were fixed and treated with an antibody against the major surface glycoprotein (MSG) of Pneumocystis conjugated to Texas Red. Slides were analyzed using fluorescent microscopy. Slides were analyzed for attachment of organisms to macrophages using percent attachment (percent of macrophages with at least one organism attached) and attachment index (average number of organisms attached to a macrophage) measures.

Results:
Following a 2h Pc-Alveolar Macrophage binding assay at 4°C, 41% attachment was achieved and green fluorescence was not observed among these macrophages. However, when this binding assay was performed for 2h at 37°C, 84% attachment was achieved and 82% of macrophages demonstrated green fluorescence.

Slight increases in green fluorescence were observed after one additional hour at 37°C among macrophages in both the 4°C and 37°C groups. A corresponding decrease in both percent attachment and the attachment index were observed following this 1h 37°C incubation between both experimental groups.

When Pc was attached at 4°C no green fluorescence was observed, however after an additional 4 hours at 37°C, 79% of macrophages fluoresced green.

Conclusions:

The results demonstrate that the green fluorescence observed in macrophages treated with Bodipy labeled Pc is indicative of phagocytosis. No fluorescence was observed among macrophages incubated at 4°C, a temperature previously shown to allow attachment while inhibiting phagocytosis of Pc. However experiments at 37°C result in green fluorescence, which increases in time dependent manner. Furthermore, organisms that were attached at 4°C, were subsequently phagocytosed when placed at 37°C in a time dependent manner. This novel assay technique will be useful for future investigations into the interactions between Pc and alveolar macrophages and may prove useful in other disease models.