

Destruction of Lung Surfactant of Alveolar Epithelial Cells through *Pneumocystis jiroveci* Infection

by
Daniel Schwerin

College of Medicine Class of 2007
Medical University of South Carolina
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Abstract

Pneumocystis jiroveci is a well known opportunistic infective microbial organism that causes Pneumocystis pneumonia (PCP) in immunocompromised humans. *P. jiroveci* had a name change in 1999 from *P. carinii* after DNA analysis confirmed that it was a fungal microbe that infected *Homo sapiens*. Alveolar epithelial cells from the host interact through specific glycosphingolipids on the cellular membrane called lipid microdomains or “rafts”. CDw17 receptors found within lactosylceramides interact with the β -glucans of *Pneumocystis jiroveci* and initiate the immune response that causes the accumulation and activation of macrophages within the lung parenchyma. Having an increase in the number of macrophages within the lung tissue causes the destruction of lung surfactant which in turn can bring about the mortality of the patient infected with the *Pneumocystis* fungal organism.

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Author’s Addresses:

dlschwerin@alumni.musc.edu

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Introduction

Over the past hundred years, a little organism that is now known to cause many problems in the human population was originally discovered. At first this microorganism was classified as a protozoan agent, but recently was transferred to the kingdom fungi. *Pneumocystis jiroveci*, initially identified in 1909 by Chagas as a trypanosome, was later brought into the microbial morphology by the science community as *Pneumocystis carinii* (Stringer, Beard, Miller and Wakefield / 2002). Up until 1988 this organism was still being classified as a protozoan, but with the ability to purify and analyze deoxyribonucleic acids, *Pneumocystis*

carinii was transferred from the protozoan family of microbes to that of a fungal lineage (Bartlett / 2002). Otto Jirovec, a Czechoslovakian parasitologist, was the first person to indicate that there was a difference between the different types of *Pneumocystis* organism that infected animals versus humans (Stringer et al / 2002). As far back as 1976, Dr Frenkel had initiated steps to switch the name of *Pneumocystis carinii* to that of *Pneumocystis jiroveci* in accordance to the observation by Jirovec (Stringer et al / 2002). It was not until 1999 that the scientific community made the switch from the species name *carinii* to that of *jiroveci*, in honor of Otto Jirovec, and thus made a distinct classification in the organisms that

infect humans versus other animal species (Stringer et al / 2002).

Pneumocystis jiroveci, the fungal microbe that invades *Homo sapiens*, is an opportunistic infection of immunocompromised hosts. Most commonly associated nowadays with the pathogen that cause autoimmune deficiency syndrome (AIDS), *Pneumocystis jiroveci* was also seen in the 1950's in malnourished children in orphanages in Iran (Stringer et al / 2002). Walzer indicates that through out the world most healthy people have antibodies for *P. jiroveci* (Walzer / 1999). Having antibodies to *P. jiroveci* would indicate exposure to this infectious fungal organism occurring in early childhood (Walzer / 1999).

P. jiroveci is comprised of a number of different lipid moieties that helps the organism survive in a very hostile environment, i.e. the human body. The interaction of the phospholipids, sterols, quinines and sphingolipid components of *P. jiroveci* (Kaneshiro / 1998) with different cell lines in the human allows for both growth and development of the organism as well as destruction to the human lung parenchyma (Hahn, Evans, Kottom, Standing, Pagano and Limper / 2003). *P. jiroveci* actual mechanism for invasion of the host cells is through the use of its lipid components and the lipid receptors on the host (Mañes, del Real and Martínez / 2003).

Lipid microdomains

Human cellular membranes contain a number of different molecules that form a barrier against the introduction of intracellular pathogens. Within the phospholipid bilayer there is an abundant, but specific area called a lipid-microdomain or lipid raft (Ha, Kwak, Lee, Kim and Lee / 2003). Iwauchi and Nagaoka (2002) indicate that the glycosphingolipid components are comprised of hydrophobic

ceramide lipid and hydrophilic sugar moieties. More specifically these membranes contain glycosphingolipids, cholesterol, and proteins which include glycosylphosphatidylinositol (GPI)-anchors (Ha et al / 2003). The importance of these individual lipid components with the bound proteins allow for the transmission of cellular signals through cell membrane receptors (Ha et al / 2003). Individually, the components of the cellular membrane can not activate or recruit other components within the cytoplasm or phospholipid bilayer (Mañes et al / 2003). When the glycosphingolipids, cholesterol, proteins and sugar moieties coalesce, they produce the larger domains which allows for the trafficking and signal-transduction, which is evident through cellular proliferation, migration, adhesion or apoptosis (Mañes et al / 2003). Of the independent molecules, cholesterol is said to be the compound that 'glues' the lipid raft together and removal of this 27 carbon structure would cause the demise of the cellular microdomains (Mañes et al / 2003).

Evidence of the importance of the cellular lipid rafts is shown by the body being able to regenerate, carry on communication between extra and intracellular compartments and sustain life. Microorganisms also use these microdomains in order to gain entrance into individual cells by activating specific receptors and thus infecting target cells (Mañes et al / 2003). Mañes et al (2003) further discuss the importance of "hijacking" the microdomain is that bacteria and other microbial organisms prefer to enter through the rafts. Several important reasons that bacteria enter through the lipid-microdomains are: avoiding intracellular degradative pathways that would be harmful to the individual bacteria and also by activating the cell signaling system that leads to ruffling and rearrangement of the cytoskeleton within the

cell membrane (Mañes et al / 2003). Rearranging the cytoskeleton is necessary for the individual microbes to hijack the lipid raft and thus enter the target cells without being identified by the host immune system (Mañes et al / 2003).

Lipid receptors

Excluding cholesterol, the other major form of glycosphingolipids found within the cell membrane is a lactosylceramide-enriched glycosphingolipid. The purpose of this specific glycosphingolipid is that it is found within the cell signaling component of the cellular membrane (Iwabuchi and Nagoka / 2002). Initially, Hahn et al (2003) report that there is an interaction between the β -glucans of *Pneumocystis carinii* (pneumocystis strain that infects rats and mice) and the β_2 integrin of CD11b/CD18 receptors of human immunologic cells, primarily neutrophils and macrophages (Iwabuchi and Nagoka / 2002). Within the neutrophils and macrophages the lactosylceramides function in the generation of superoxides that help in the destruction of pathogens (Iwabuchi and Nagoka / 2002). Research performed by Hahn et al shows that CDw17 receptors found within the lactosylceramide of alveolar epithelial cells interact with specific β -glucans receptors on pneumocystis microbes (Hahn et al / 2003). So the importance of the CD11b/CD 18 receptors found within the cellular membranes of macrophages and neutrophils take a back seat in the activation of the immune system when *Pneumocystis jiroveci* infects human alveolar cells.

The human immune system is very adept at destroying invading pathogens when all of the individual components are working properly. Problems arise when specific pieces in the immune system are missing or are in short supply. *Pneumocystis jiroveci*, being the opportunistic organism that it is, is able to respond to individuals with weak

immune systems by invading the individual's respiratory system. Within the lung parenchyma, pneumocystis interacts with the CDw17 receptors found within the microdomains or lipid-rafts of alveolar epithelial cells (Hahn et al / 2003).

In their study of rat alveolar epithelial cells, Hahn et al show that the interaction between the β -glucans of *Pneumocystis carinii* and the lactosylceramide lipids found within the CDw17 receptors of the lipid rafts to activate macrophage inflammatory protein-2 (MIP-2), the counterpart found within *Homo sapiens* is human CXC chemokine IL-8 (Hahn et al / 2003). This interaction between the CDw17 receptors and the β -glucans is important because it is one of the first times shown that cells outside of the immune system play an integral part in the activation of the glucan-induced inflammatory signaling in lung epithelial cells during *Pneumocystis carinii* infection (Hahn et al / 2003). The establishment of infection by the *P. carinii* organism is by the binding of the microbe with the alveolar epithelial cells, thus creating the activation of the MIP-2 protein, a potent stimulant of neutrophil's accumulation and activation (Hahn et al / 2003).

Having an outside source as the activator for an immune response is not always the best of situations. The interaction between the β -glucans and CDw17 receptors on alveolar epithelial cells activate the neutrophils and consequently the macrophages to infiltrate the lung parenchyma. Severe *P. carinii* pneumonia per Hahn et al is characterized by lung inflammation secondary to intense neutrophilic activity which in turn leads to impaired gas exchange, diffuse alveolar damage, respiratory failure and possibly death (Hahn et al / 2003). The high mortality rate of immunocompromised patients with an infection from *Pneumocystis jiroveci* is not from the organism itself, but through the

inflammatory response secondary to the activation of lung macrophages (Hahn et al / 2003).

Lung surfactant

The only piece of information that is found within the literature on the possible cause of mortality in humans is the destruction of lung surfactant by the *Pneumocystis jiroveci* organism (Wright, Notter, Wzng, Harnesn and Gigliotti / 2001). Lung surfactant is comprised of dipalmitoyl phosphatidyl choline and functions to reduce the surface tension of fluids within the lung and prevent collapse of the lungs during end expiration phase of breathing (Berg, Tymoczko and Stryer / 2001). The cause of destruction of lung surfactant by the *P. jiroveci* microbe is through several independent pathways. First and foremost, *P. jiroveci* is a single celled organism that has a phospholipid bilayer that is comprised of lipids from de Novo formation within the microbe itself, and other parts are scavenged from the host that the *P. jiroveci* microbe is invading (Kaneshiro / 1998).

The second part in the destruction of surfactant is the interaction of the mannose receptors found on macrophages to the Glycoprotein-A of *Pneumocystis carinii* (O'Riordan, Standing and Limper / 1995). Problems arise because lung surfactant protein-A has a similar mannose-binding protein (Wright et al / 2001). Interaction between surfactant protein-A (SP-A) at the mannose receptor site and surfactant protein-D at a different site allows for *P. jiroveci* to control and ultimately use the lipids within these surfactants as a part of its own chemistry (Wright et al / 2001). The human immunodeficiency virus impairs the mannose receptor function on macrophages against *P. carinii* (Wright et al / 2001).

Conclusions and Future Directions

So one would have to ponder, there is an increase in the number of macrophages within the lung parenchyma secondary to the induction of human CXC chemokine IL-8 through the CDw17 receptors of alveolar epithelial cells by the β -glucans of *Pneumocystis jiroveci*. Macrophages are unable to interact with *Pneumocystis jiroveci* because the HIV virus is impairing the mannose receptors on the macrophages. Lung surfactant protein-A has a homologous mannose-binding protein that is in contact with *P. jiroveci*, but has a potential of coming into contact with free macrophages within the lungs. The function of macrophages is to phagocytize and destroy compounds that it comes into contact, i.e. pulmonary lung surfactant in this case. In essence it appears that *P. jiroveci* is activating our own immune system to destroy the lung surfactant that it needs in repairing and regenerating more microbial organisms.

Pneumocystis Pneumonia (PCP) has been a wide spread problem with an increase in the number of cases of human immunodeficiency virus, malnutrition in primarily third world countries and other immunocompromising conditions (Stringer et al / 2002). Through DNA analysis researchers have been able to identify that *Pneumocystis jiroveci* is not a protozoan, but rather a fungal micro-organism that creates major havoc on the pulmonary system of immunocompromised individuals (Stringer et al / 2002). The fact that *P. jiroveci* plays only a small integral part in the activation of the host immune system and destruction of lung surfactant is quite amazing at the amount of damage that is done to the pulmonary system.

Hahn et al (2003) indicate that the use of nonspecific anti-inflammatory therapies help in the decrease of mortality during Pneumocystis pneumonia. Reason for the use of corticosteroid treatment during

Pneumocystis pneumonia is to limit the number of macrophages that enter the lung parenchyma during time of infection from *P. jiroveci*. As previously discussed, Hahn et al (2003) showed through research that the induction of MIP-2 during infection from *P. carinii* had a devastating increase in the number of macrophages in an area surrounding the alveolar epithelial cells. These macrophages would subsequently destroy not the *P. jiroveci* that they are suppose to, but in turn would break down the surfactant being produced by the lung tissue. Furthermore an increase in macrophage activity in the lung tissue would also increase the amount of inflammation in that area and cause secondary problems such as hypoxemia or acidosis. In the end, humans, through the immune system, are our worst enemy during times of infection from *Pneumocystis jirovecii*.

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