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Slicing through the challenge of maintaining *Pneumocystis* in the laboratory

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ABSTRACT *Pneumocystis jirovecii* is a major fungal pathogen of humans that causes life-threatening lung infections in immunocompromised individuals. Despite its huge global impact upon human health, our understanding of the pathobiology of this deadly fungus remains extremely limited, largely because it is not yet possible to cultivate *Pneumocystis in vitro,* independently of the host. However, a recent paper by Munyonho et al. offers a major step forward (F. T. Munyonho, R. D. Clark, D. Lin, M. S. Khatun, et al., 2023, mBio 15:e01464-23, https://doi.org/10.1128/mbio.01464-23). They show that it is possible to maintain both the trophozoite and cyst forms of the mouse pathogen, *Pneumocystis murina,* in precision-cut lung slices for several weeks. Furthermore, they demonstrate that this offers the exciting opportunity to examine potential virulence factors such as possible biofilm formation as well as antifungal drug responses in the lung.

KEYWORDS *Pneumocystis*, fungal pathogenicity, medical mycology, precision-cut lung slices

Pneumocystis jirovecii is a deadly pathogen of humans that causes life-threatening pneumonia in immunocompromised individuals. This fungus is found in the lungs of infants (1, 2) and is known to cause fatal pneumonia in patients with compromised immune responses, including transplant recipients (3, 4). It continues to be one of the most prevalent and severe infections among individuals with HIV/AIDS (5), representing a significant health challenge, particularly within the developing nations of sub-Saharan Africa. In addition to HIV/AIDS, a variety of other factors that promote immunosuppression have emerged as significant risk factors for *Pneumocystis* pneumonia (PCP) (6, 7). Moreover, PCP is becoming increasingly problematic for non-HIV-infected individuals in developed countries, including Sweden (8), the United Kingdom (9), and the United States (10). Despite the major impact of *P. jirovecii* on global health, our understanding of the biology of this fungus remains alarmingly limited. This is largely because significant challenges, primarily the lack of robust *in vitro* and *ex vivo* models, have severely restricted the experimental dissection of the pathogenesis of *Pneumocystis* species. Indeed, the absence of robust cultivation techniques is widely acknowledged to be the major obstacle in *Pneumocystis* research. Thus far, attempts to bypass likely auxotrophic requirements (predicted on the basis of genome sequence analyses) through supplementation of culture media have not proven sufficient to facilitate growth *in vitro* (11).

Therefore, the paper entitled "Precision cut lung slices as an *ex vivo* model to study *Pneumocystis murina* survival and antimicrobial susceptibility" represents an exciting step forward (12). In this paper, Munyonho et al. describe the development of an innovative *ex vivo* model, based on the use of precision-cut lung slices (PCLS) from mice, to study the survival of the mouse pathogen *Pneumocystis murina*. Using a combination of cellular, molecular, and histochemical approaches, they firstly confirmed the viability of the lung slices over a 15-day period. Their thin lung sections maintained

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the structural and functional characteristics of lung tissue, thereby providing a physiologically relevant environment for the pathogen by replicating the alveolar space of the host lung where *Pneumocystis* predominantly resides (13). Then, using a combination of reverse transcriptase-quantitative polymerase chain reaction and immunohistochemistry, Munyonho et al. demonstrated that, over this period, both the cyst and trophozoite forms of the fungus retained their viability in these lung slices but not in Dulbecco's modified Eagle's medium alone. This was the case for lung slices from immunocompetent (C57BL/6) as well as immunodeficient (*Rag2*$^{-/-}$ *Il2rγ*$^{-/-}$) mice. Having established this ground-breaking *ex vivo* PCLS system, the authors then highlight its potential applications by examining the susceptibility of *P. murina* to commonly used antifungal treatments such as trimethoprim-sulfamethoxazole and echinocandins (14, 15).

This important paper represents a significant breakthrough for the *Pneumocystis* research community and medical mycologists at large. From an academic perspective, the observation of Munyonho et al. that *P. murina* forms fungal aggregates, potentially biofilms, in the tissue slices is intriguing. This observation, which is consistent with previous reports (16), paves the way toward the experimental dissection of this phenomenon as well as the mechanisms by which *Pneumocystis* species adhere to and scavenge from host tissue. Some limitations, such as the lack of a systemic immune response in the tissue slices, will influence their utility in the dissection of antifungal immune responses. Nevertheless, the native lung environment and the heterogeneity of resident cells are preserved in PCLS. For example, alveolar macrophages and alveolar dendritic cells are critical for the immune response against *Pneumocystis* (17, 18), and imaging of these cells in lung slices reveals similar antigen uptake and presentation when compared with *in vivo* imaging techniques such as intravital stabilized lung imaging (19). Looking forward, the use of PCLS in conjunction with advanced microfluidics and live imaging is likely to facilitate studies of the dynamics of immune recognition of *Pneumocystis* while reducing animal usage. Transcriptomic analyses of infected PCLS are likely to provide complementary information about *Pneumocystis* pathobiology and immune activation (20). The growth of *Pneumocystis* on PCLS might even empower forward genetics to yield mutants capable of independent growth on appropriately supplemented media *in vitro*.

Precision-cut tissue slices can also be prepared from organs such as the brain, liver, and pancreas, as well as from different species including rodents, monkeys, and humans. Indeed, in several recent studies, PCLS from a variety of species, including human, have been used to elucidate the adherence of other microbial pathogens and local cytokine responses against these pathogens (21–24). Therefore, from a clinical perspective, this new paper adds to the recent successful application of *ex vivo* PCLS systems for modeling lung disorders (25, 26) and for studies of asthma (27), chronic obstructive pulmonary disease (COPD) (28), and idiopathic pulmonary fibrosis (29). Organotypic brain slice cultures have also been used successfully to study neuroimmune responses to brain infections with *Cryptococcus neoformans* (30), a fungal pathogen that usually infects the lung initially but later disseminates to cause injury to the brain. *Pneumocystis* infections of the central nervous system have been reported in patients with advanced HIV disease (31, 32), but these remain understudied. Therefore, the development of organ-specific approaches for *Pneumocystis* also provides a unique opportunity to study fungus-host interactions in extra-pulmonary infections. Also, the availability of surgically resected lung and brain tissue offers exciting translational opportunities to use precision-cut tissue slices to study human-specific host-pathogen interactions, as well as to streamline the evaluation of new antifungal agents, thereby accelerating the pace of therapeutic advancements.

In summary, the study by Munyonho et al. represents an exciting step forward in *Pneumocystis* research. The development of a viable *ex vivo* model using PCLS will not only enrich our understanding of *Pneumocystis* biology but will also potentially shape how we approach PCP treatment and prevention.

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