ICCC2026

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28 June - 3 July 2026 | University of Exeter, UK

POSTER ABSTRACT BOOK

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Poster Abstract Book

International Cryptococcal Conference 2026

Exeter, UK | June 29 – July 3, 2026

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Poster Abstracts

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SESSION 1

1 – Operationalising Community Engagement as an Implementation Strategy for HIV-Associated Fungal Infection Research: The IMPRINT Multicountry Model

Kennedy Mupeli

Molloy SF, Edkins L, Lawrence D, Mupeli K, Jarvis J, Harrison T, Govender N., Falconer J.

Botswana Harvard Health Partnership

Background: HIV-associated fungal infections such as cryptococcal meningitis remain a significant cause of AIDS-related deaths in low- and middle-income countries. Despite the availability of effective diagnostics and treatments, their impact is limited by barriers including delayed presentation, low treatment literacy, and insufficient involvement of patient perspectives. The IMPRINT consortium uses structured community engagement to enhance the acceptability and relevance of interventions for HIV-associated fungal infections. By addressing behavioural and health system factors affecting cryptococcal infection management, IMPRINT bridges the gap between host and pathogen interaction. Methods: IMPRINT applied a standardised community engagement approach in seven countries across Africa and South East Asia, forming Community Advisory Boards (CABs) as governance bodies. Multilingual facilitation and treatment literacy tools supported CAB activities, which included protocol reviews, feedback sessions, and qualitative data collection. Knowledge gains were measured with pre- and post-training questionnaires, and engagement outcomes were tracked through protocol amendments and implementation changes. Regional workshops further strengthened capacity in governance and planning. Results: CAB members showed improved understanding of cryptococcosis, research processes, and patient rights. Community feedback led to protocol updates, including better consent processes and tailored screening and treatment. Outputs included advocacy briefs and stakeholder dialogues supporting access to diagnostics and therapies. Capacity strengthening improved CAB workplans and coordination, enhancing research acceptability and feasibility. Conclusions: IMPRINTs measurable engagement strategy proves effective in multinational HIV-associated fungal infection research. Integrating patient perspectives improves intervention relevance and delivery, supporting responsive research and implementation in high-burden regions.

2 – Validation of the FungiXpert Cryptococcal Capsular Polysaccharide K-Set Lateral Flow Assay

Julia Palmucci

Asmus Tukundane (2), Wilber Bakka (2), Dora Babirye (2), Mercy Amanige (2), Caleb Skipper (3), David Meya (2), and David Boulware (3)

Center for Global Health and Social Responsibility, University of Minnesota, Minneapolis, MN, USA

Background:

Cryptococcal antigen (CrAg) testing is important for the screening and early diagnosis of cryptococcal meningitis. There is only one CrAg testing kit with FDA approval, the CrAg lateral flow assay (LFA) by Immy. Here we analyze the diagnostic performance of the FungiXpert Cryptococcal Capsular Polysaccharide K-Set LFA by Genobio Pharmaceutical.

Methods:

We tested 296 cryopreserved plasma and serum samples and 96 cryopreserved CSF samples collected from 392 participants between 2020 and 2025. Individuals had HIV seropositivity and were enrolled in two outpatient cohorts with CD4<200 cells/mcL in Uganda, and one hospitalized cohort of suspected meningitis in Kampala, Uganda. FungiXpert CrAg LFA testing was conducted in accordance with manufacturers instructions and read by three blinded observers. The IMMY CrAg LFA test served as reference standard.

Results:

The FungiXpert CrAg LFA had a sensitivity of 96.8% [91/94; 95%CI 91.0-99.3] in CSF. In serum/plasma the FungiXpert CrAg LFA had a sensitivity of 95.9% [188/196; 95%CI 92.1-98.2%] and specificity of 99% [99/100; 95%CI 94.6-100%]. When compared to the results of the IMMY Semi-Quantitative CrAg LFA, all false negatives occurred in samples classified as low titer. Adjusting for the cryptococcal antigenemia prevalence in Uganda of 5.7%, the FungiXpert CrAg LFA had an 85.3% positive predictive value and 99.8% negative predictive value in serum/plasma.

Conclusion:

We found that the FungiXpert kit had a high specificity and suboptimal sensitivity compared with the IMMY CrAg LFA. Reduced sensitivity in low-titer samples highlights the need for robust evaluation, including specimens from persons with early or asymptomatic infection.

3 – Birth and clinical outcomes of pregnant women with Cryptococcal Antigenemia in outpatient clinics in Uganda

Elizabeth Nalintya

Elizabeth Nalintya[1], Alexandra Poeschla[2], Ann Fieberg[2], Patricia Nerima[1], Edith Ajilong[1], Tessa Adzemovic[2], Faridah Nakiganda[1], Elli Schwartz[2], Biyue Dai[2], David Meya[1], David R Boulware[2], Radha Rajasingham[2]

1 Infectious Diseases Institute, Makerere University College of Heath Sciences; 2 University of Minnesota

Introduction: Clinical trials for the treatment and prophylaxis for cryptococcal meningitis have historically excluded pregnant persons. As a result, clinical and pregnancy outcomes for pregnant persons with HIV-associated cryptococcal infection are unknown. We evaluated clinical and pregnancy outcomes among pregnant participants with cryptococcal antigenemia. Methods: We prospectively enrolled adults with advanced HIV disease (defined as CD4 ≤200 cells/μL) within the ENCORE clinical trial (NCT05085171). Enrollment is ongoing at 10 of the 26 participating outpatient HIV clinics in Uganda. We present an analysis of baseline characteristics, opportunistic infections, birth outcomes, and clinical outcomes among pregnant participants. Results: Between May 2022 and April 2025, 114 pregnant participants with advanced HIV disease were enrolled, of whom 45% were antiretroviral therapy naive, median CD4 cell count was 124 cells/μL, and 42% were in their second trimester. Pregnant participants were less likely to be CrAg positive (2% vs. 7%, p=0.023) and less likely to be TB LAM positive (11% vs. 28%, p<0.001) compared with non-pregnant female participants. One CrAg-positive pregnant participant was enrolled at eight weeks gestation, received fluconazole for 24 weeks and delivered at term. The other participant was CrAg SQ 3+ at 16 weeks gestation, received 5 doses of weekly Amphotericin B deoxycholate (1mg/kg) without fluconazole, and delivered at term. Conclusions: Among pregnant participants with advanced HIV disease, prevalence of cryptococcal antigenemia was 2%, significantly lower than that of non-pregnant participants. Antifungal therapy was used with favourable maternal outcomes.

4 – Cost of cryptococcal meningitis hospitalization among adults with advanced HIV disease in Uganda

Elizabeth L. Schwartz

Elizabeth K. Nalintya (2), Patricia Nerima (2), Ruth E. Ajilong (2), Ann M. Fieberg (3), Biyue Dai (3), David B. Meya (2), David R. Boulware (1), Bruce Larson (4), Radha Rajasingham (1)

Division of Infectious Diseases and International Medicine, Department of Medicine, University of Minnesota, Minneapolis, MN, USA.

Background: Cryptococcal meningitis is a leading cause of hospitalization and death among people with advanced HIV disease. We estimated the cost of cryptococcal meningitis hospitalization among adults with advanced HIV in Uganda. Methods: We conducted a micro-costing analysis among adults with advanced HIV hospitalized for cryptococcal meningitis within a trial (NCT05085171) conducted in Kampala, Uganda (May 2022January 2025). Participant-specific resource use (length of stay, antifungal medications, and treatments for co-occurring conditions) was extracted from case report forms. Standard inpatient resource use (personnel, supplies, diagnostics, and lumbar punctures) was estimated using hospital-level data and national treatment guidelines. Costs were summarized in 2025 US Dollars (USD). Results: Among 1,429 outpatients with advanced HIV, 22 (1.5%) were hospitalized for cryptococcal meningitis during six-month follow-up. Median length of stay was 11 days (IQR 715). Median total cost of hospitalization was 969 USD (IQR 6471366). Antifungal medications accounted for the largest proportion of total costs. Specifically, 47% of hospitalization costs were attributed to flucytosine, and 15% were attributed to liposomal amphotericin B. Personnel salaries (15%), inpatient bed costs (7.4%), and monitoring labs (6.9%) contributed smaller shares of total cost. Conclusions: Cryptococcal meningitis hospitalization imposes a substantial financial burden on the Ugandan healthcare system, with costs dominated by flucytosine. The average cost of a cryptococcal meningitis hospitalization is comparable to screening over 270 people for cryptococcal antigenemia, highlighting the potential for early screening and pre-emptive treatment to reduce mortality and healthcare system costs among people with advanced HIV.

5 – Baseline CMV Viraemia and Neurocognitive Performance Among Cryptococcal Meningitis Survivors

Nsangi Laura Joan

Jayne Ellis, Nicole Engen, Mahsa Abassi, Caleb P Skipper, ALice Namudde, Grace Menya, David R Boulware, David B Meya

Infectious Disease Institute, Makerere University, Kampala, Uganda

Background: CMV viraemia is highly prevalent among adults with advanced HIV disease and is associated with increased mortality. We sought to investigate the association between baseline CMV viraemia and neurocognitive performance among Ugandan adults with HIV-associated cryptococcal meningitis. Methods: We enrolled a prospective cohort of Ugandans with cryptococcal meningitis between August 2021 and April 2024, and quantified baseline CMV viremia via polymerase chain reaction on cryopreserved plasma. We assessed neurocognitive function among survivors at 3 and 12 months using a composite quantitative neurocognitive performance z-score (QNPZ). Results: Among 223 survivors diagnosed with HIV-associated cryptococcal meningitis, 26% (57/223) had baseline CMV viraemia. At enrollment, individuals with CMV viraemia presented with significantly lower CD4 count 16 [IQR 6,47] vs. 25 [IQR 9,70] cells/mm3, lower cerebrospinal fluid (CSF) white blood cell count 4 [IQR4,65] vs. 45 [IQR 4,130] cells/mm3, and lower CSF protein 52 [IQR 29,100] vs. 100 [IQR 40,138] mg/dL. There was no difference in overall neurocognitive performance (QNPZ-8) at 3 or 12 months between participants with vs. without baseline CMV viraemia. Individuals with CMV viraemia had similar QNPZ-8 scores compared with those without CMV viraemia at 3 months -0.9 [IQR -01.2, 0.5] vs -0.9 [IQR -1.3, -0.4], p=0.62and 12 months -0.5[IQR -0.7, 0.0] vs. -0.6 [IQR -0.9, -0.1], p=0.22. Conclusion: In HIV-associated cryptococcal meningitis, individuals with baseline CMV viraemia demonstrated reduced baseline CSF inflammatory responses; however, this did not translate into differences in neurocognitive performance at 3 or 12 months post cryptococcal meningitis diagnosis among survivors.

6 – AUTOPSY FINDINGS IN PERSONS DIAGNOSED WITH HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS.

Morris Kyozo Rutakingirwa

Carol O. Namujju 1, Jeya Sushimitha 4, Masumba J. Dalton 1,2, Jolive Nakayemba 1,2, Richard Kwizera 1, Samuel Okurut 1, Robert Lukande 1,2, Mawanda Anatoli 1,3,5, David R. Boulware 7, David B. Meya 1,6,7 and Melanie R. Nicol 8.

Infectious diseases institute, College of health sciences, Makerere University, Kampala Uganda

Introduction: Data on autopsy findings among persons with cryptococcal meningitis (CM) remain limited, particularly in advanced HIV disease. Methods: We conducted autopsies among consented participants with CM enrolled in a prospective cohort in Kampala, Uganda from January to September 2025. Autopsy findings were correlated with perimortem clinical records from hospitalization. Results: Twenty-four autopsies were reviewed; 58% (14/24) were female. Median (IQR) age was 40 years (3046), and median CD4 count was 5 cells/L (025). One cadaver had a gravid uterus (20 cm) with a single deceased fetus, and one was 18 days postpartum. All cadavers demonstrated brain pathology, including hazy meninges (n=24) consistent with meningitis, features of elevated intracranial pressure (n=18) with edema, narrow sulci and flattened gyri; parenchymal necrosis (n=4), herniation (n=3), and hemorrhage (n=2). All also had pulmonary pathology, including edema (n=24), consolidations (n=17), pleural effusions (n=2), collapse (n=1), cavities (n=1), and granulomas (n=1), although only 37.5% (9/24) had a perimortem diagnosis of respiratory distress or aspiration pneumonia. Lymphadenopathy was present in 33% (8/24), with cryptococcal cysts identified histologically in one case. Renal pathology was common (17/24), including cryptococcal cysts (n=9), focal segmental glomerulosclerosis (n=7) , and caseating granulomas (n=1). Although 29% (7/24) had antemortem diagnoses of tuberculosis, only one case was histopathologically confirmed. Aspergillosis and Kaposis sarcoma were identified in one case each. Conclusions: Beyond extensive CNS involvement, asymptomatic pulmonary, renal, and lymphatic pathology is common in HIV-associated cryptococcal meningitis. Antemortem presumptive tuberculosis diagnoses frequently lacked histopathologic confirmation. Safe feeding practices are needed to reduce aspiration.

7 – Headache as a predictor of meningitis in ambulatory patients with symptomatic HIV-associated cryptococcal antigenemia

Maria Sarah Najjuka

Alexandra Poeschla2, Abduljewad Wele3, Elizabeth Nalintya1, Paul Kirumira1, Peruth Ayebare1, Grace Nakitto1, Fred Turya1, Lydia Nankungu1, Caleb Skipper2, Biyue Dai3, David Boulware2, David Meya1,2 Radha Rajasingham2

Infectious Diseases Institute, College of health Sciences, Makerere University, Uganda

Background: People with HIV-associated cryptococcal antigenemia are at high risk for meningitis and death. In resource-limited settings, lumbar puncture to evaluate for meningitis is infrequently performed in asymptomatic individuals; therefore, symptomatic individuals are prioritized. We evaluated the predictive value of meningeal symptoms for cryptococcal meningitis in people with cryptococcal antigenemia. Methods: We conducted a secondary analysis from three randomized clinical trials in Uganda. We included adults with meningeal symptoms who had cerebrospinal fluid (CSF) cryptococcal antigen (CrAg) testing performed. Logistic regression and classification trees were used to determine the associations between meningeal symptoms and meningitis. Area under the Receiver Operating Characteristic curve (AUROC) and misclassification rate were used to evaluate model performance. Results: Among 344 participants, median CrAg titer was 1:1280 (IQR 1:100,1:2560). Overall, 285 (83%) participants presented with headache and 205 (60%) had meningitis. Presence of headache was associated with meningitis (adjusted odds ratio 11.7, 95% Confidence Interval [CI] 5.2328.50). The AUROC of headache alone to predict meningitis was 0.65 (95%CI 0.610.69), with 95% (195/205) sensitivity and 35% (49/139) specificity. The AUROC improved to 0.86 (95%CI 0.820.90) when stiff neck, photophobia, confusion, sex, and CrAg titer >1:160 were added to the model. In the final classification tree, headache combined with CrAg titer >1:160 had the highest probability (79%) of meningitis. Conclusions: Headache and high CrAg titer are the most reliable predictors for meningitis. In the absence of plasma CrAg titration, symptoms of headache, stiff neck, photophobia, and confusion predict meningitis for individuals with cryptococcal antigenemia.

8 – False Positive IMMY Serum Cryptococcal Antigen Lateral Flow Assay Results Due to Improper Use of Titration Buffer.

Asmus Tukundane

Wilber Bakka1, Tadeo K Kiiza1, Mercy Amanige1, Dora Babirye1, Timothy Mugabi1, Abdu Musubire1, David B Meya1,3, David R Boulware3, Brian Doherty4, Richard Kwizera1, Caleb P Skipper3.

Makerere University Infectious Diseases institute -Kampala Uganda.

Background: False-positive results with the IMMY cryptococcal antigen (CrAg) lateral flow assay (LFA) may result from procedural or reagent-related errors. Although the CrAg LFA is a reliable diagnostic tool for Cryptococcus, occasional false positives complicate interpretation, highlighting the need to identify their causes to maintain accuracy and avoid unnecessary treatment. Immunological factors such as antigen cross-reactivity, rheumatoid factor, or heterophilic antibodies, and technical errors including incorrect use of titration diluent, improper dilution, or use of the positive control as diluent, may lead to false-positive outcomes. Methods: Two patients who were initially reported as CrAg positive by a peripheral laboratory were referred to Kiruddu Hospital in Kampala, Uganda, for confirmatory testing. Repeat tests were conducted using specimen diluent following IMMY manufacturers protocol, as well as incorrectly using titration diluent only. Semi-quantitative CrAg LFA testing and control assays were performed to identify the source of error. Results: Repeat patients serum testing with the correct specimen diluent yielded negative results, whereas testing with only the titration diluent produced false positive bands. Serial dilutions confirmed persistent false positivity up to 1:80 using titration diluent, while all dilutions using specimen diluent remained negative at 1:5 titration. Conclusion: Incorrect use of titration diluent instead of specimen diluent can lead to false positive CrAg LFA results due to the absence of the blocking agent that neutralizes heterophilic antibodies. Technical laboratory error may be the cause of false positive results. Removal of titration diluent in situations where CrAg titers are not performed may improve real-world diagnostic performance.

9 – Phospholipid homeostasis in host-Cryptococcus interactions during pulmonary cryptococcosis

Chaoyang Xue

Siddhi Pawar, Robert Tancer, Yina Wang, Varsha Gadiyar, Raymond Birge

Rutgers University

Cryptococcus neoformans is the leading cause of life-threatening fungal meningitis. Macrophages are the first line of defense against Cryptococcus infection, yet the molecular mechanisms underlining macrophage-Cryptococcus interaction remains incompletely understood. Our previous study demonstrated that loss of lipid flippase function by deleting CDC50 gene in C. neoformans leads to increased accumulation of exocytoplasmic phosphatidylserine (PS) on the plasma membrane, enhanced phagocytosis and macrophage killing, resulting in avirulence in animal infection models. Notably, this PS-driven phagocytosis occurs independently of the host PS receptor MertK. Our recent findings reveal that cdc50 cells produce significantly more extracellular vesicles (EVs) with elevated surface PS levels compared to wild-type H99. Both cdc50 cells and their EVs exhibited increased production of multiple phospholipid species, including PS and PE, as shown by our lipidomic analysis. Furtherm ore, supplementing H99 culture with cdc50 EVs during macrophage-H99 interaction markedly increases in H99 phagocytosis, indicating that cdc50 EVs can enhance the fungicidal activity of macrophages. Collectively, our data demonstrate that fungal lipid homeostasis is crucial for C. neoformans resistance to macrophage recognition and clearance during early host-pathogen interactions in cryptococcosis. Lipid flippase function emerges as a potential drug target, and we have developed a peptide-based inhibitor that shows potent activity against Cryptococci.

10 – Mortality among individuals with subclinical cryptococcal meningitis identified through cryptococcal antigen (CrAg) screening programs in South Africa and Tanzania

Sile Molloy

Molloy SF,1,2 Rundogo J,2 Berghammer-Bohmer R,2 Thombrayil A,2 Nel J,3 Tsitsi M,4 Verhagen D,5 Bremer M,5 Kabanda D,5 Njabulo M,5 Maphalala L,6 Ferdinand R,7 Rutananukwa N,7 Mosses A,7 Makoko N,7 Edkins L,8 Fatti I,9 Van Wyk T,10 Adams J,1 Halley-Stott R,10 Eriksson M, 2 Meiring S,11Quan V,11Mashau R,12 Lawrence DS,2,13,14 Mupeli K,14 Meintjes G,10 Wilson D,9 Moosa MYS,8 Jaffar S,15 Loyse A,1 Wang D,16 Bradley J17, Murphy K,2,10 Boyles T,3 Mfinanga S,7 Lesikari S,7 Jarvis J,13,14 Harrison TS,1,18,19 Govender NP,1,2,12,18* Wake R,1,2,19* and the EFFECT trial consortium

City St George's, University of London

Background Up to 40% of blood cryptococcal antigen (CrAg)-positive individuals have subclinical cryptococcal meningitis (CM), yet clinical outcomes for this understudied subgroup remain unclear. While current guidelines recommend intensive treatment for all individuals with CM, evidence is needed to inform risk-stratified management strategies. Methods In South Africa and Tanzania, we prospectively followed-up 2 cohorts of adults with CD4 counts <100 cells/l: 1) subclinical CM (CSF CrAg-positive) and 2) symptomatic CM. Baseline characteristics and outcomes over 6 months were recorded. Results From November 2022 to October 2025, 62 adults with subclinical CM and 364 with symptomatic CM were enrolled. Median age was 39 years. Seven days of amphotericin B plus flucytosine was the most commonly administered induction treatment in both cohorts (71% and 67%, respectively). Subclinical CM cohort: 68% (42/62) were ART-experienced. Median baseline opening pressure (OP) was 15cmH2O (IQR: 11-27, n=32). Two-week, 10-week and 6-month mortality was 8.1% (95%CI: 2.7-17.8, 5/62), 35.5% (95%CI: 23.7-48.7, 22/62) and 40.3% (95%CI: 28.1-53.6, 25/62), respectively. Symptomatic CM cohort: 58% (201/344) were ART-experienced. Median baseline OP was 28cmH2O (IQR: 18-40, n=78). Two-week, 10-week and 6-month mortality was 21.7% (95%CI: 17.6-26.3, 79/364), 40.9% (95%CI: 35.8-46.2, 149/364) and 44.5% (95%CI: 39.3-49.8, 162/364), respectively. Discussion The majority of opening pressures were normal at baseline among the subclinical CM patients, with fewer CM-related deaths than in the symptomatic cohort, as indicated by a lower 2-week mortality. However, 10-week and 6-month mortality was similar between the cohorts. Alternative risk-stratified management approaches may be considered for these patient groups.

11 – Cryptococcosis drives hospitalisation and death in advanced HIV disease in Uganda

Patricia Nerima

Radha Rajasingham, Elizabeth K. Nalintya, Ruth E. Ajilong, Faridah Nakiganda, Alexandra Poeschla, Joseph Musaazi, Elli Schwartz, Ann Fieberg, Biyue Dai, David Meya, David Bouwlare

Research department, Infectious Diseases Institute, Makerere University, Kampala, Uganda

Background: Among people with HIV, 30% present with advanced HIV disease (defined as CD4 200 cells/L). Causes of hospitalisation, duration, and characteristics of those hospitalised are poorly described among people with advanced HIV disease. Methods: Among adults with advanced HIV disease enrolled into a cluster-randomised trial evaluating opportunistic infection screening and prophylaxis for people with advanced HIV disease (NCT05085171), we evaluated participants who were hospitalised during study participation. Participants were enrolled across 26 outpatient HIV clinics in Uganda from May 2022 to January 2025. Baseline characteristics, length of hospital stay, final diagnoses, and clinical outcomes were summarised descriptively. Fishers Exact Test was used to compare proportions. Results: Among 1429 participants with advanced HIV disease, 83 (6%) were hospitalised, with a median hospital stay of 7 days (IQR 414). Hospitalised individuals had a median CD4 cell count of 50 cells/L (IQR 22-91 cells/L), and 68% were ART-naive at enrollment. Overall, 40% (33/83) of hospitalised participants were plasma cryptococcal antigen (CrAg) positive at enrollment compared with 5% of non-hospitalised participants ($p<0.0001$). Hospitalised participants were more likely to be TB LAM positive (37% vs 27%, $p=0.0569$) and receiving active TB treatment (57% vs 38%, $p=0.0007$) compared with non-hospitalised participants. Most common causes of hospitalisation were TB (23/83; 27%) and cryptococcosis (22/83; 26%). Overall, 20% (17/83) died during hospitalisation, and an additional 13% (11/83) died within 30 days post-discharge. Conclusions: CrAg-positive individuals are at substantially higher risk for hospitalisation, and Cryptococcosis is a leading cause of hospitalisation among people with advanced HIV disease.

12 – Whole genome alignment of Cryptococcus and Kwoniella reveals patterns of conservation

Sosie Yorki

Christina Cuomo

Brown University, Department of Computational Molecular Biology

Fully characterizing the evolution of pathogenicity in Cryptococcus neoformans is crucial for identifying more effective drug targets. Ortholog analysis of Cryptococcus and Kwoniella have pinpointed genes unique to pathogenic Cryptococcus, but evolution of non-coding regions remain less well understood. A recent GWAS analysis identified SNPs in non-coding regions linked to higher fungal burden in patients infected with C. neoformans, demonstrating the potential for non-coding variation to regulate pathogenicity functions. To investigate C. neoformans at nucleotide-level resolution, we aligned the reference genomes of 31 species with Progressive Cactus and used the Phylogenetic Analysis with Space/Time Models toolkit to find patterns among evolutionarily conserved and accelerated regions in Cryptococcus and Kwoniella. Close to 75% of bases in C. neoformans conserved across the whole tree belonged to coding sequence. Within protein-coding genes, exons varied in degree of cons ervation depending on its position relative to UTRs. Some intergenic regions and ncRNAs were also highly conserved, indicating these non-coding regions serve an important function. Examining sequence unique to pathogens, we found over 30% of non-protein coding bases in C. neoformans were exclusively aligned across all pathogenic species. This suggests newly evolved regulation of pathogenesis-conferring processes. The highest rates of evolutionary acceleration were seen in intergenic regions, 5 UTRs, and 3 UTRs (in descending order). Future analyses will re-examine transcriptional data to identify potential genes within conserved intergenic regions and improve exon-intron boundaries in pathogenic Cryptococcus.

13 – Cryptococcus neoformans Sequence Type 93 is Associated with Reduced Type-1 Immune Responses in Ugandan Patients with HIV-associated Cryptococcal Meningitis

Kisakye Diana Kabbale

1David Meya, 2Gerald Mboowa, 3Thomas Kono, 4Segun Fatumo, 5Kirsten Nielsen

Infectious Disease Institute

Cryptococcus neoformans causes fatal meningitis commonly among patients with advanced HIV disease in Sub-Saharan Africa. The relationship between C. neoformans genotypes and virulence is incompletely understood. We performed whole-genome sequencing (WGS) on 493 C. neoformans isolates from Ugandan patients with HIV-associated Cryptococcal Meningitis (HIV-CM) enrolled in the ASTRO (NCT01802385) and AMBITION (MREC 1297) clinical trials conducted between 2015 and 2020. Multiple Locus Sequence Types (ST) were extracted from the WGS data. Associations between ST and virulence using clinical, microbiological, and immune-phenotype data were assessed using Chi-square tests for categorical phenotypes and the Wilcoxon rank-sum test for continuous phenotypes. Of the analysed isolates, 42.2% (208/493) were from patients who died, whereas 57.8% (285/493) were from patients who survived. Thirty-four (34) unique STs were identified among the HIV-CM clinical isolates. ST93 and non-ST93 acco unted for 65.1% (n=321) and 32.5% (n=160) of the C. neoformans isolates, respectively, while 2.4%(n=12) of the isolates could not be typed. There was no statistically significant difference in mortality, nor in known CM prognostic factors such as Glasgow coma scale, fungal burden, CD4+ T cell counts, cerebrospinal fluid (CSF) white blood cell count, CSF protein, and CSF glucose between patients infected with ST93 isolates and those infected with non-ST93 isolates. However, patients infected with ST93 isolates expressed significantly lower levels of the Type-1 proinflammatory cytokines. Since Th1 immune responses are essential for fungal clearance from the central nervous system, this finding potentially explains the high prevalence of ST93 among immunosuppressed patients with advanced HIV-CM in Uganda.

14 – Subtelomeric Genomic Rearrangement underlies Phenotypic Variation and Amphotericin B Resistance in Clinical isolates of Cryptococcus neoformans

Sujiraphong Pharkjaksu

Yun C. Chang, Kyung J. Kwon-Chung

Molecular Microbiology Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland, United States of America

Emergence of amphotericin B (AmB) resistance remains uncommon in Cryptococcus but is increasingly recognized and poses a serious clinical threat, as AmB-based regimens remain a cornerstone of induction therapy for cryptococcal meningitis. We analyzed serial clinical isolates of C. neoformans obtained from a patient with treatment-failed cryptococcosis before and after AmB-based treatment. The post-treatment isolate exhibited elevated AmB MICs (23 g/ml) compared with the pre-treatment isolate (0.250.38 g/ml). Phenotypic assays showed that AmB-resistant isolates had reduced growth at elevated temperature and impaired fitness under membrane perturbation, redox/oxidative stress, and iron limitation. Consistent with altered redox homeostasis, fluorescence-based assays revealed increased basal reactive oxygen species in cytosolic and mitochondrial compartments in the resistant isolate. To define the underlying mechanism, we performed RNA-seq comparing genome-sequenced w ild-type and post-treatment isolates. Gene Ontology enrichment identified ergosterol biosynthesis as the most significantly altered pathway, with marked downregulation of ERG2 in post-treatment isolates. Long-read nanopore sequencing uncovered an approximately 20-kb terminal deletion on chromosome 1 encompassing ERG2, followed by de novo telomeric repeat addition consistent with chromosome-end healing. Complementation of AmB-resistant isolates with wild-type ERG2 restored phenotypic defects and AmB susceptibility, confirming ERG2 loss as the primary driver of AmB resistance. These data demonstrate a clinically relevant route to AmB resistance emergence in C. neoformans mediated by chromosome-end loss and telomere addition and highlight ERG2-dependent sterol homeostasis as critical for core physiology and polyene susceptibility.

15 – Phased nuclear and mitochondrial genome assemblies of Cryptococcus A/D hybrids using long-read sequencing and trio-binning

Nicolas Helmstetter

Rahul Anand, Qinxi Ma, Diana Tamayo, Rhys A. Farrer.

University of Exeter

Hybridisation between Cryptococcus lineages is common in both environmental and clinical settings and has been linked to altered virulence and antifungal susceptibility. However, the highly heterozygous, mosaic genomes of A/D hybrids are challenging to assemble and phase, limiting our understanding of their genome architecture and evolution.

Here we generated Oxford Nanopore long-read data for four Cryptococcus A/D hybrids and used a hifiasm trio-binning strategy informed by A and D parental references. Reads were assigned to the A- or D-derived haplotype and assembled separately, yielding two highly contiguous, near-complete haploid assemblies per hybrid.

We also identified mitochondrial contigs and processed them with Circlator to generate circularised mitochondrial genomes of ~2933 kb with gene content and organisation consistent with previously described Cryptococcus mitogenomes.

These data provide a detailed view of how hybrid genomes are structured and reshaped following hybridisation and demonstrate that combining Nanopore long reads with trio-binning enables robust phasing of Cryptococcus hybrid genomes. These assemblies provide a resource for dissecting the genetic and mitochondrial basis of hybrid fitness, adaptation and pathogenesis in Cryptococcus.

16 – The cell-type specific neuroimmune response to cryptococcal infection of murine hippocampal brain slices

Alison Gifford

Emily Higgitt 1, 2, 3, Maahir Kauchali 1, 2, 3, Amalia Awala 1, 2, 3, 5, Anja de Lange 1, 2, 3, Lilitha Cengani 1, 2, 3, Ivy Dambuza 4, Dorit Hockman 2, 5, Teresa Steyn 2, 5, Joshua Selfe 2, 5, Adilia Warris 4, *Joseph Raimondo 2, 5 and *Rachael Dangarembizi 1, 2, 3, 4

University of Exeter

Cryptococcal meningitis (CM) is often associated with severe neurological damage but the neuropathophysiology of this fatal disease is incompletely understood. This study aims to characterise the immunomodulatory transcriptional activity of each cell type in the murine brain to C. neoformans using single-nuclei RNA sequencing (snRNAseq). Hippocampal organotypic brain slices were prepared from six-day old mouse pups and stimulated with either 1 x 108 C. neoformans cells, lipopolysaccharide (LPS) or normal growth media for 24 hours. Brain slices were Dounce homogenised and a single nuclei suspension recovered for sequencing. Libraries were generated by 10X Genomics and sequenced via Illumina. Microglia did not significantly upregulate any major pro- or anti-inflammatory genes in cryptococcal infection, in contrast to the pro-inflammatory gene upregulation with LPS treatment. There is no clear phenotype for cryptococcal-treated microglia, but upregulated genes include phagocytic receptors with anti-inflammatory properties including Colec12 and Lrp1. Astrocytes have more significantly upregulated pro-inflammatory genes in response to Cryptococcus including Stat3, Nos2, Cxcl2, Fos and Nfkb1. Astrocytes role at the blood-brain barrier is also affected with upregulated Vegfa and Piezo1. Tnfaip2, critical in tunnelling nanotube biogenesis, is upregulated in both astrocytes and microglia. Excitatory neurons have the highest number of differentially expressed genes in response to Cryptococcus with upregulated Stat3, Nfkb1, Fos and Il4. The glutamate cycle is also affected with downregulated Gls and Grik2. This data suggests that microglia are phagocytosing Cryptococcus leading to a minor anti-inflammatory response. Astrocytes are significantly pro-inflammatory and, along with excitatory neurons, have a significant Stat3-response to Cryptococcus.

17 – Methodological Considerations for Flow Cytometric Analysis of Polyploidy in Cryptococcus neoformans

Ashley H. Lim

Claudia A. Petrucco, Huarui Zhou, Steven B. Haase

Duke University

During infection, Cryptococcus neoformans can form large polyploid cells called Titan cells. To better understand how Titan cells form, we have been examining in vitro conditions that can drive polyploidy. A classical method for quantitating cell ploidy is to stain DNA followed by flow cytometric analysis; however, we have discovered that culturing conditions and staining procedures can influence fluorescence measurements in a way that does not appear to accurately reflect DNA content. In some cases, these conditions can produce data that falsely indicate the presence of polyploid cells. Using our substantial experience in analyzing polyploidy in S. cerevisiae, we have designed new flow cytometry protocols to reliably identify true polyploid cells in C. neoformans. We compared the accuracy of our new protocols with published PI/DAPI protocols to assess the benefits and limitations of different DNA dyes. Our results highlight the high sensitivity of DNA fluorescence to norma l and stress culture conditions, as well as to fixing and staining parameters for C. neoformans. Here, we present the comparative analysis and our most optimized DNA staining protocol. We define conditions that contribute to an increase in fluorescence in normal haploid cells and make recommendations for validating polyploidy in the cell population.

18 – Dissecting the role of Mad1 in Cryptococcus neoformans

Wulfrun Godson

Kevin Hardwick, Thom Davies

Hardwick Lab - University of Edinburgh

The spindle assembly checkpoint (SAC) protects cells against chromosome segregation defects by delaying anaphase until all chromosomes are correctly bioriented. Mad1 is a key scaffolding protein involved in this checkpoint. Though found on kinetochores during mitosis, Mad1 is localised at the nuclear periphery during interphase and pull down experiments have shown that this may be due to binding with TPR, a component of the nuclear pore complex (NPC). It is possible that this increases the robustness of the SAC by enabling Mad1 to produce important SAC effectors early at the nuclear periphery during, so they are ready for the onset of mitosis. Mad1 deletion mutants (mad1) therefore show a strong checkpoint defective phenotype, being unable to delay mitotic progression when treated with anti-microtubule drugs. When induced to form titans, these mutants also show a significant reduction in titan cell viability, suggesting that Mad1 is required for their survival and/or di vision. During this project, Cryptococcus neoformans mutants expressing truncated Mad1 were used to show that the first 100 residues of the N-terminal tail are necessary for nuclear periphery localisation during interphase, and therefore may contain a TPR-interacting motif. Interestingly, these mutants were checkpoint defective but had no titan cell viability defects, suggesting that Mad1s titan cell role is independent of both its checkpoint function and its TPR interaction. I hope that this project will be able to shed some light on this additional, as yet uncharacterised, function of Mad1 and increase our understanding of the mysterious titan cell mechanisms.

19 – Co-culture with Mycobacterium tuberculosis causes remodelling of Cryptococcus gattii morphology and cell wall architecture

ZAR NI SAN

Hilaire Irere, Ivy M. Dambuza, Liliane Mukaremera

Medical Research Council Centre for Medical Mycology at the University of Exeter, University of Exeter, EX4 4QD, United Kingdom; School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, Whitla Medical Building, Belfast, BT9 7BL, United Kingdom

Cryptococcus species complex, consisting of Cryptococcus neoformans and Cryptococcus gattii, and Mycobacterium tuberculosis (MTB), are opportunistic pathogens associated with high mortality in individuals with weakened immune systems. Evidence indicates that in areas where both pathogens are endemic, the prevalence of co-infections has increased significantly. Moreover, co-infections with Mycobacterium tuberculosis significantly increase mortality rates to 22%, compared with 18% for Cryptococcosis alone. Therefore, this study aimed to investigate how exposure to Mycobacterium tuberculosis influences the morphogenesis and cell-surface architecture of Cryptococcus gattii. We analysed morphological attributes of Cryptococcus gattii (cell body diameter, capsule thickness, and cell wall composition) during co-culture with Mycobacterium tuberculosis. Then, the results were compared with those obtained for Cryptococcus gattii in monoculture. Our results reveal that when Cryptococcus gattii is grown in a physiologically relevant human plasma-like medium, the cell body diameter and capsule thickness increase with rising MTB concentration. Notably, the proportion of titan cells also increased significantly from 6.5% (Cryptococcus gattii monoculture) to 16.6% of the total cell population in co-culture. Capsule thickness was also significantly elevated in the presence of MTB. At the highest MTB dosage, MTB induced cell wall remodelling, with increased mannan and decreased chitin levels when Cryptococcus gattii was co-cultured with MTB. In conclusion, Mycobacterium tuberculosis transforms Cryptococcus gattii into a titan-dominant, thicker-capsulated phenotype, accompanied by cell wall modifications. These changes could enhance fungal persistence in co-infected patients and underscore the urgent need for targeted diagnostic methods and therapies for co-infections.

20 – Regulation of extracellular vesicle biosynthesis by the TOR pathway in the fungal pathogen Cryptococcus neoformans

Isabelle MOUYNA

Julien Bazile(1), Patricia Flamant(1), Pierre-Henri Commere(2) and Guilhem Janbon(1)

RNA and Extracellular Vesicles Unit, Institut Pasteur, Inserm U1359 Mycologie Moléculaire et Médicale, Paris, FRANCE

Extracellular vesicles (EVs) are small structures surrounded by a lipid bilayer that are released by cells into the extracellular environment. They are involved in many biological processes including intercellular communication, antifungal resistance and virulence. In fungi, EVs were first isolated in Cryptococcus and have since been described in other human pathogenic fungal species. They contain lipids, polysaccharides, pigments, proteins and RNAs, but the exact composition and function of these cargo molecules remains largely unknown. Unlike in mammals, the mechanism by which Cryptococcus neoformans EVs are secreted out from the cells is unknown. Recently, the structural and compositional aspects of Cryptococcus EVs have been described and their potential role as a vaccine has been suggested. However, the biosynthesis pathways that regulate EV production in fungi have yet to be described. We investigated the dynamics and genetics of Cryptococcus EV biogenesis by screening a library of kinase mutant strains for EV production. We demonstrated that EV release is regulated differently during cellular growth phases and identified mutants associated with different cellular pathways that affect EV production. Three of these kinases, PKH1, YPK1 and SCH9, which are related to sphingolipid biosynthesis showed a drastic reduction in EV production as well as in the estimated size distribution of the vesicles and their protein profiles. Their exact role in EV production is under investigation. Key words: extracellular vesicles, kinases, TOR pathway

21 – Structure-function analysis of the RNAi machinery in Cryptococcus

Finn Ritchie

Rob van Nues, Jeyaprakash Arulanandam, Elizabeth Bayne

University of Edinburgh

RNAi is a conserved mechanism of gene regulation in eukaryotes. Central to the pathway are the Argonaute proteins which, using small RNAs as guides, act to recognise and silence specific targets. In Cryptococcus, the primary targets of the RNAi machinery are the transposable elements. Loss of the RNAi mechanism in Cryptococcus has been associated with increased antifungal resistance and pathogenesis. Beyond a role in transposon regulation, however, the mechanisms and wider functions of RNAi in Cryptococcus remain poorly understood. We aim to characterise the proteins involved in Cryptococcus RNAi, with a particular focus on the essential complexes such as the Argonaute-containing RNA-induced silencing complex (RISC): the molecular machinery responsible for mediating silencing. Initial sequence alignments and structural modelling of the Cryptococcus Argonaute proteins in AlphaFold3 have revealed an insert rich in RGG motifs, a feature uncommon in orthologs that could mediate novel interactions and/or regulation. Additionally, Argonaute immunoprecipitation followed by mass spectrometry has identified several RNAi-associated proteins whose roles in Cryptococcus remain undetermined. To characterise the functional complexes, we are purifying Argonaute proteins directly from Cryptococcus, and isolating Argonaute-containing complexes through size exclusion chromatography for analysis with mass spectrometry and cryo-EM. By investigating the components and assembly principles of the RNAi protein complexes, and probing the mechanics of RNAi-mediated silencing through structure-guided separation-of-function mutations in cells, this work will provide valuable insights into the relationship between RNAi and infectivity in Cryptococcus.

22 – Cryptococcus spore germination can occur in the absence of oxygen

Jackie Spieles

Barnes M, Hull CM

University of Wisconsin-Madison

Because invasive cryptococcosis is caused by fulminant yeast growth, Cryptococcus spores must germinate into yeast to cause disease in mammalian hosts. Our lab used a high-throughput screen of small molecules to identify novel germination inhibitors. We discovered one class of compounds that presumably targets complex II of the electron transport chain (ETC) in a fungal-specific manner. Although yeast (obligate aerobes) cannot grow in the presence of ETC inhibitors or in anaerobic environments, we discovered that spores can germinate in the absence of detectable oxygen. This calls into question the need for ETC function and the mechanism of complex II inhibitors during germination. These paradoxical findings reveal a substantial knowledge gap in how respiration and metabolism operate during the energy-intensive process of germination. To evaluate the role of the ETC in germination, we tested inhibitors of ETC complexes and conducted transcriptomic analyses on spores germina ting hypoxically. Our data indicate that ETC function is important for hypoxic germination, including a role for the cyanide-independent alternative oxidase (AOX1). Future experiments will determine the unique and potentially targetable mechanisms of cellular respiration in germinating Cryptococcus spores, including evaluating potential roles of non-oxygen terminal electron acceptor pathways.

23 – Defining Mechanisms of Cryptococcal Sterol Transport

Dong-gyu Kim

Tamara L. Doering

Washington University in St. Louis

The critical membrane lipid ergosterol must distribute between its site of synthesis and key membranes. Specialised lipid transport proteins coordinate the movement of sterols and other lipids at membrane contact sites, sub-30 nm spaces between adjacent membranes. These proteins represent an unexplored aspect of cryptococcal ergosterol biology that may reveal pharmacologically exploitable processes. Accordingly, we sought novel or understudied proteins that influence cryptococcal sterol homeostasis. To do this we screened for spontaneous mutants that suppress the growth defects of cells lacking the sterol transporter Ysp2. Whole genome sequencing of suppressors revealed numerous nucleotide polymorphisms in coding regions directly relevant to sterol biosynthesis, regulation, and transport; this strongly validated the utility of our screen for studying sterol homeostasis. Excitingly, one unstudied gene encodes a protein with a predicted phospholipid ligand-binding domain, a f eature linked to lipid transporters. We hypothesised that this protein affects sterol transport indirectly, possibly through a mechanism involving a phospholipid ligand. Subsequent characterization revealed roles of the gene in lipid homeostasis, cell morphology, stress tolerance, and virulence, while microscopy suggests the encoded protein localises at ER-plasma membrane contact sites. Further, in vitro studies of the exogenously expressed protein suggest a non-sterol lipid ligand. These results support our working model that this novel protein associates with membrane microdomains enriched with its lipid ligand and influences sterol transport through a non-sterol transport mechanism. Current studies aim to further define its role in sterol transport and potentially inhibit its activity. Overall, this work will illuminate the fundamental process of sterol transport.

25 – Investigating the role of Cyclins in the Cryptococcus neoformans cell cycle

Tymoteusz Cezary Pacamaj

C-author affiliations:

University of Edinburgh

Many mechanisms underpinning the cell-cycle of Cryptococcus neoformans remain poorly understood; for example, the role that cyclins and cyclin dependent kinases play in its regulation. While these proteins have been identified and have known homologues in other fungi, their functions have not been characterised in Cryptococcus. My project, therefore, focuses on investigating the cyclins Cln1, Clb2, and Clb3 and Cdk1 (a cyclin dependent kinase) to which cell-cycle checkpoints that they regulate and the mechanisms underpinning this regulation. In other fungi, homologues of these factors have been shown to be involved in both the G1/S checkpoint and the G2/M checkpoint However, the study of Cdk1 and Clb2 are complicated by the fact that they are essential. I will use an auxin degron system which allows for the conditional degradation of these proteins from cells. This, combined with studying cellular DNA content via flowcytometry, allows us to see which checkpoints these cyclins are involved in by determining what percentage of the population is 1C or 2C following degradation. Furthermore, in the related fungi Ustilago maydis, the homologue for Clb3 is a significant virulence factor due to its role in ploidy changes and morphogenesis. Since Titan cell formation, a key cryptococcal pathogenic process, remains mechanistically uncharacterised but is known to involve changes in ploidy and morphology, I will investigate if the removal of Clb3 impairs this. Titan cell formation has been linked to cyclins previously, with Cln1 knockouts upregulating it. Therefore, I will uncover the function of cyclins in these important mechanisms.

26 – A role for aquaporin (Aqp1) in the control of Cryptococcus neoformans cell morphology

Piotr Stempinski

Francisco G. Hernandez, J. Alberto Patiño-Medina, Isabel Jimenez, Samuel Rodrigues dos Santos Junior, Peter Agre, Arturo Casadevall

Johns Hopkins University

Aquaporins are small, integral membrane channels that facilitate the transport of water across cellular membranes and, in the case of aquaglyceroporins, can also conduct specific neutral solutes, such as glycerol. These proteins are conserved across biological kingdoms, yet their roles in fungal virulence remain relatively understudied. In Cryptococcus neoformans, an opportunistic fungal pathogen, we examined the organisms single aquaporin, Aqp1, and uncovered unanticipated influences on cellular morphology. Loss of Aqp1 resulted in smaller cells, whereas its presence promoted the formation of enlarged titan-like cells. This shift in size was closely linked to intracellular redox physiology. Consequently, the overexpression of the cryptococcal aquaporin increased sensitivity to oxidative stress and led to the largest titan-like cells; antioxidant supplementation suppressed this enlargement, consistent with a ROS-dependent regulatory mechanism. Additionally, Aqp1 overexpr ession produced vacuolar abnormalities in titan-like cells, suggesting that excessive water influx strained intracellular organization during rapid cell expansion. These findings position Aqp1 at a functional crossroads connecting membrane transport, oxidative balance, and size control, and they support a model in which an aquaporin contributes to the morphological plasticity that allows C. neoformans to adapt to environmental pressures.

27 – Mechanism of antifungal persistence of Cryptococcus neoformans against Amphotericin B

Ruchi Agrawal

Hugo Varet, Etienne Kornobis, Adrien Pain, Aude Sturny-Leclére, Alexandre Alanio

Institut Pasteur, Paris

Cryptococcus neoformans can survive in the host during antifungal treatment, leading to treatment failure and increased clinical burden. Unlike resistance, which involves stable genetic changes, antifungal persistence does not include any genetic changes. Yet antifungal persisters survive during antifungal therapy. Our project aims to understand the antifungal persistence phenotype of C. neoformans against Amphotericin B (AMB). We found that at a 16-fold minimum inhibitory concentration (MIC) of AMB, most of the stationary-phase cells die (60-90%), but a small subpopulation of persister cells survives. Surprisingly, we saw cell division even at such a high concentration of the drug. For the first time, we successfully isolated persister cells and revealed many interesting and distinct phenotypes. Persisters are smaller in size, with a larger central vacuole, fewer mitochondria and many super-sized lipid droplets. Cell walls chitin and beta-glucan, cell membranes ergos terol and polyunsaturated fatty acids are also modified in persisters to adapt to the stressful environment. To decrease the oxidative stress generated by AMB, persisters upregulate the expression of genes involved in antioxidation and downregulate the expression of genes involved in mitochondrial respiration. Persisters also have upregulated polyamines and trehalose pathways as a protective mechanism to fight antifungal stress. Persister cells have lower expression of virulence markers, which could help them stay unnoticed inside the host for a longer time, which will be explored further with host interaction studies. Overall, our study has highlighted previously unknown and uncommon mechanisms that cryptococcal persister cells use to protect themselves during antifungal treatment.

28 – A microfluidic approach to capture the morphological switch between yeast and titan cells in Cryptococcus neoformans

Iana Kalinina

Abigail Potton, Connor Kirke, Darren Thomson, Sandra Catania, Elizabeth R Ballou

MRC Centre for Medical Mycology, University of Exeter, UK

Fungi undergo changes in shape in response to a variety of environmental stimuli. Cryptococcus neoformans is a pleiomorphic fungus that offers a particularly dynamic example of this capacity. While in nutrient replete conditions it proliferates as a uniform population of budding yeast. However, upon infection of the human lung, haploid yeast can convert to large, polyploid titan cells. These cells enable immune evasion, alter immune response, drive drug resistance, and are associated with poor patient outcomes. Titan cells are characterised by a thick cell wall, large central vacuole, and robust capsule, as well as their increased size and ploidy. Although the existence of titan cells has been well documented in patient samples and can be replicated using simple in vitro culture conditions, it has remained a challenge to capture the formation of this morphotype in real time. Time lapse microscopy using open culture wells is hampered by the propensity of vacuolarising cells to float, coupled with the impact Brownian motion that causes non-adherent cells to drift out of frame. At the same time this fungus raises many questions, the answer to which depends on being able to image single cells over extended periods. Microfluidics allows us to trap single cells for days, keep them under highly controlled conditions mimicking the host environment, and perform time lapse imaging, including following perfusion with chemical compounds like antifungals. Using this approach. we observed dynamic processes like titan cell formation, kinetochore dynamics, and nuclear dynamics during cell division over 24 hours, and here report our observations of this dynamic process. This approach opens new avenues for research on Cryptococcus neoformans.

29 – Cryptococcus neoformans and Cryptococcus gattii exhibit distinct neuroimmune responses in the CNS microenvironment

Lilitha Cengani

Anja de Lange1,2,3, Maahir Kauchali 1,2,3, Timothy D Reid4, Rachael Dangarembizi1

Division of Physiological Sciences, Department of Human Biology, Faculty of Health Science, University of Cape town, Cape Town, South Africa 2Neuroscience Institute, Groote Schuur Hospital, University of Cape Town, Cape Town, South Africa

Cryptococcus neoformans (Cn) and Cryptococcus gatti (Cg) are the primary causative agents of cryptococcal meningitis, a life-threatening neuroinfectious disease affecting both immunocompromised and immunocompetent individuals. While Cn has global distribution, Cg, previously thought to be found in tropical regions has been identified in previously non-endemic regions. Despite this, very few studies have investigated species-specific mechanisms that drive infection in the microenvironment of the central nervous system (CNS). Thus, we investigated species-specific host-pathogen interactions using hippocampal organotypic brain slice cultures (OBSCs).

Hippocampal OBSCs were made from post-natal day 7 C57BL/6 mice and maintained for six days in vitro. Thereafter, slices were treated with a GFP-expressing reporter strain of Cn or Cg and assessed 24-hours post infection for fungal burden, immune cell activation and inflammatory responses using immunofluorescence staining and cytokine profiling. Preliminary findings revealed a significantly higher fungal burden of Cn compared to Cg. Immunofluorescence staining was performed to identify microglia expressing nuclear transcription factor, NFIL-6. Quantitative analyses showed no differences in the proportions of NFIL-6 expressing microglia in response to Cn when compared to untreated OBSCs but a marked increase in Cg. Moreover, cytokine analysis revealed a treatment-dependent fold change of a subset of analytes with Cn associated with interleukin (IL)-13 and Cg with RANTAS (CCL5) chemokine. These findings suggest Cn and Cg exhibit distinct immunomodulatory mechanisms of infection in the brain that warrant further investigation and may potentially inform differences in clinical outcomes. Ongoing flow cytometric analysis are being used to analyse responses in greater detail.

30 – Human Brain Organoids: A New Model to Study Cryptococcus neoformans Neurotropism

Hannah Brown Harding

Alfred Harding (1,2), Lee Gehrke (1,2), Jay Vyas (3,4,5)

Division of Infectious Diseases, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA; Department of Medicine, Harvard Medical School, Boston, MA, USA; Broad Institute of MIT and Harvard, Cambridge, MA, USA.

With the rise in immunocompromised individuals and patients with immune-related comorbidities such as COVID-19, the rate of fungal infections is growing. This increase, along with the current plateau in antifungal drug development, has made understanding the pathogenesis and dissemination of these organisms more pertinent than ever. The mouse model of fungal infection, while informative on a basic scientific level, has severe limitations in terms of translation to the human disease. Here we present data supporting the implementation of the human cerebral organoid model, which is generated from human embryonic stem cells and accurately recapitulates relevant brain cell types and structures, to study fungal infection and dissemination to the central nervous system (CNS). This approach provides direct insight into the relevant pathogenesis of specific fungal organisms in human tissues where in vivo models are impossible. With this model system we assessed the specific brain tr opisms and cellular effects of fungal pathogens known to cross the bloodbrain barrier (BBB), such as Cryptococcus neoformans. We determined the effects of this fungal pathogen on the overall gross morphology, cellular architecture, and cytokine release from these model organoids. Furthermore, we demonstrated that C. neoformans penetrates and invades the organoid tissue and remains present throughout the course of infection. These results demonstrate the utility of this new model to the field and highlight the potential for this system to elucidate fungal pathogenesis to develop new therapeutic strategies to prevent and treat the disseminated stages of fungal diseases such as cryptococcal meningitis.

31 – Differential activation and exhaustion of circulating T-cells and monocytes at baseline may determine risk of developing cryptococcal meningitis among individuals with HIV-associated asymptomatic cryptococcal antigenemia

Mark Oilor

Eric Katagirya, Elizabeth Nalintya, Caleb P Skipper, Annie Wilt , Paul Kirumira, Allan Buzibye, Sandra Naluyima, Abduljewad Wele , Biyue Dai , David R Boulware , Radha Rajasingham, David B. Meya , & Samuel Okurut .

Makerere University

Introduction: The role of host immune factors in the progression of asymptomatic cryptococcal antigenemia (CrAg) to cryptococcal meningitis outcomes are unclear. This study assessed immunological predictors of progression to meningitis despite fluconazole preemptive therapy. Methods: Twenty-one Ugandans adults living with HIV with asymptomatic CrAg and titers >=1:160 received fluconazole preemptive therapy. Six developed meningitis while 15 did not, surviving to 6-months without meningitis. Baseline peripheral blood mononuclear cells were analyzed via spectro-flow cytometry. Plasma fluconazole was measured by mass spectrometry at week 4. Results There were no significant differences in age, CrAg titers, CD4 counts, fluconazole concentrations (median 36 mcg/mL with meningitis vs 22 mcg/mL without meningitis), ($p>0.05$). Individuals who developed meningitis had lower %CD4+ T-cells, higher %CD8+ T-cells, and lower CD4/CD8 ratio (0.12 versus 0.20, $p=0.020$) than those without men ingitis. The %CD69 and CD163 activation marker expression on classical monocytes was lower in individuals who developed meningitis than those without ($P=0.018$; $P=0.008$, respectively). The %CCR4 expression on CD4+ T-cells, reflecting Th2 chemokine receptor, was higher in individuals with meningitis than those without. Individuals who developed meningitis had higher %PD-1 expression (immune cell exhaustion marker) on CD4+ T-cells , CD8+ T-cells, and on classical monocytes ($P<0.05$ for each). The CXCR3+/CCR4+ (Th1/Th2) ratio on CD4+ T-cells was lower among individuals with meningitis than those who without (0.635 versus 1.043, $p=0.007$), reflecting a greater Th2>Th1 skew. Conclusions: Lower CD4/CD8, Th1/Th2 ratios, divergent activation, and increased T-cell/monocyte exhaustion may increase the risk of developing meningitis among individuals with HIV-associated asymptomatic cryptococcal antigenemia

32 – Characterization of monocyte-derived microglia-like cells as an in vitro model of Cryptococcus-microglia interactions.

William Newton

Dr Elizabeth Ballou, Dr Carolina Coelho, Dr Jonathan Witton, Dr Rachael Dangarembizi

University of Exeter

Background and aims: Cryptococcus neoformans is a neurotropic fungal pathogen that causes lethal meningitis in immunocompromised individuals. Within the central nervous system, C. neoformans can use microglia, the resident macrophages of the brain, as a reservoir of infection and subvert their immune functions. However, microgliaCryptococcus interactions remain poorly characterised, partially due to limitations of murine models and immortalised microglial cell lines. Monocyte-derived microglia-like cells (MDMLs) provide a tractable primary human alternative. Here, we aimed to characterise MDML responses to C. neoformans and assess their suitability as an in vitro model of cryptococcal meningitis. Methods: Human peripheral blood mononuclear cells were isolated by density gradient centrifugation, monocytes enriched by attachment, then differentiated into MDMLs using IL-34 and GM-CSF over 14 days. Differentiation was confirmed by immunocytochemistry. MDMLs were infected with serum-opsonised C. neoformans (H99E), and phagocytosis a were assessed by immunocytochemistry. Results: MDML differentiation was confirmed by increased expression of the microglial markers Iba1 and TMEM119 and morphological changes consistent with microglial identity, compared to donor-matched monocytes. In a pilot experiment, MDMLs efficiently phagocytosed C. neoformans, with a proportion of phagocytic cells comparable to iPSC-derived microglia, but with significantly higher fungal uptake per cell. Conclusions: Future experiments will robustly describe immunological phenotypes of MDMLs to C. neoformans via immunocytochemistry, ELISAs, and microbial proliferation assays. These will inform the potential of MDMLs as a robust and scalable human in vitro model for studying microglialfungal interactions, enabling mechanistic dissection of cryptococcal neuroimmune evasion.

33 – Differential recognition of C. neoformans by male and female human macrophages elicits contrasting immune responses

Erin McClelland

Mackenzi Lawson, Rebecca Troutman, Luke Horner, Savanna Gray, Mercedes Andrade, Abner Chen, Phoenix Davis, Basil Mustaklem, Liz Pradhan, Simran Sandhu, Jonathan W. Lowery, Sarah A. Justice

Marian University Wood College of Osteopathic Medicine

Cryptococcus neoformans (Cn) is a pathogenic yeast and the cause of cryptococcosis, a life-threatening fungal disease. Strikingly, ~70% of patients that develop cryptococcosis are male. Here, we investigated decreased Cn recognition by male macrophages and whether that affected the subsequent innate immune response. Human PBMCs were isolated from healthy male and female donors, differentiated into macrophages, and tested for immune response differences. Cn incubated with female serum showed increased binding of the opsonin serum amyloid P, possibly explaining why female macrophages show increased Cn phagocytosis. After infection, cytokine analysis showed increased secretion of proinflammatory cytokines at 24 h in male macrophages that were substantially decreased by 48 h. In contrast, female macrophages secreted fewer proinflammatory cytokines at both 24 and 48 h but had increased secretion of CCL5 (24 and 48 h) and IL-18 (48 h). These data suggested the male immune respons e was changing 24 h post-infection; thus, we tested macrophage activation state. Male macrophages were alternatively activated at 48 h, with increased secretion of arginase 1 whereas female macrophages were classically activated, with increased secretion of nitric oxide at 24 and 48 h. Cn genes related to capsule production were increased in Cn grown in male serum at 48 h. These data suggest that female macrophages better recognize Cn and become classically activated whereas male macrophages initiate an inflammatory response that is markedly reduced after 24 h, possibly due to testosterone-induced GXM secretion. Thus, antifungal therapeutics targeting testosterone-induced GXM release may improve outcomes in male patients with cryptococcosis.

34 – Unleashing C5: The complement protein antifungal role against C. neoformans

Ricardo Alexandre Frois Martins

Man Shun Fu 1 , David Lecky 1 , Rebecca Drummond 1 2

Institute of Immunology & Immunotherapy, University of Birmingham, Birmingham, UK

The cryptococcal field have used DBA2 mice as a model for C. neoformans infection, as these mice are deficient in the complement protein C5 and exhibit high susceptibility to this infection. Patients treated with C5-blocking therapy have also been reported to develop cryptococcal infections. Yet, the organ- and cell type-specific roles for C5 remain unknown although in recent years C5 have revealed to be essential for both innate and adaptive immune responses. Using a systemic (i.v.) infection approach, we observed that mice C5 deficient mice (C5-/-) had higher lung fungal burden early as day 1 post infection. Infecting mice with a C. neoformans mutant that is impaired in extracellular growth (gcs1Δ) demonstrated that poor control of pulmonary fungal burden in C5-/- mice is the consequence of impaired capacity of C5-/- to limit both extracellular and intracellular replication of the fungus. Indeed, C5-/- mice had reduced viability of lung macrophages, while bone-marrow derived macrophages from C5-/- animals had elevated intracellular growth of C. neoformans. In the brain, C5-/- and WT mice presented similar brain fungal burden throughout infection despite a significant increase in mortality and weight loss in C5-/- animals. Analysis of fungal-specific T cell infiltration and activation revealed enhanced CD4 T cell function in the C5-/- brain, consistent with greater expression of MHCII by tissue-resident microglia and exaggerated proliferation and TCR signalling by CD4 T cells when co-cultured with C5-deficient antigen-presenting cells. Altogether, we reveal a novel tissue specific mechanism of complement-dependent antifungal protection against C. neoformans.

35 – Mechanistic target of rapamycin (MTOR) signaling pathway loss-of-function variants in previously healthy patients with non-HIV cryptococcosis.

Jessica C. Hargarten

Gabriel Soares Matos2; Laura Fruhauf De Macedo2; Malcolm J. Vaughan1; Kenneth Ssebambulidde1; Guowu Hu1; Owen CR. Dean1; and Peter R. Williamson1.

Rutgers New Jersey Medical School

Cryptococcosis primarily affects individuals with advanced immunosuppression, including those with HIV/AIDS, solid organ transplants, or prolonged corticosteroid exposure; however, little is known about whether host genetic factors might predispose certain individuals to develop disease independent of known risk factors. Identification of genetic defects in human patients is an important natural experiment that can reveal important human traits required for host disease susceptibility and resistance. In the present studies, genetic analysis of a large cohort of previously healthy (PH) patients who developed infections with Cryptococcus were investigated to determine underlying immune deficiencies leading to susceptibility. In a cohort of 138 PH patients with cryptococcosis, whole exome sequencing was performed, identifying rare alleles predicted to have deleterious functional consequences (CADD >30). ToppGene pathway analysis revealed MTOR signaling pathway defects as enriched in this patient population, with 50% carrying predicted deleterious variants, including 5 in MTOR. Functional analysis showed that these MTOR variants were associated with T cell dysfunction. Mouse models of MTOR deficiency chemically (rapamycin) or genetically (CD4-Cre MTOR-floxed), exhibited increased susceptibility to cryptococcosis: reduced survival, increased fungal burdens, and diminished lymphocyte recruitment to sites of infection. Moreover, adoptively transferred MTOR-deficient CD4 T cells failed to migrate effectively during cryptococcosis. These findings are the first to identify loss-of-function MTOR variants in humans and highlight the crucial role of MTOR-related signaling in anti-cryptococcal adaptive responses. Ongoing experiments, utilizing MTOR-flox mice and human cells, aim to further elucidate MTOR's role in immunity and the unique susceptibility to this important fungal pathogen.

36 – Development and validation of an experimental evolution model for Cryptococcus neoformans during extreme temperature events.

Shannon Evans

Simon A. Johnston

School of Medicine and Population Health, Florey Institute, Bateson Centre, University of Sheffield, S10 2TN, UK

Cryptococcosis, an invasive fungal infection typically of the brain, is primarily caused by Cryptococcus neoformans, a yeast found ubiquitously in the environment. Pathogenicity of C. neoformans is thought to be a secondary effect of adapting to the environment. With climate change expected to alter the environment in various ways, there are concerns that this may drive increased virulence in fungi. Extreme temperature events have been suggested as a potential driver for virulence in fungi due to the necessity of all human fungal pathogens to tolerate mammalian endothermy. Additionally, pathways associated with virulence and antimicrobial resistance are upregulated in response to heat stress in C. neoformans. Therefore, this study has developed and validated a model for the experimental evolution of C. neoformans during extreme temperature events to determine the effects on virulence-associated traits and thermotolerance. Using a heat shock gradient, 42 degrees centigrade w as established as the highest temperature C. neoformans survived for the purpose of experimental evolution over 7 generations. Phenotypic changes associated with cryptococcal virulence were measured, and an in vitro murine macrophage model of infection was used. We successfully isolated populations with increased thermotolerance over generations, however generational viability remained low after each treatment suggesting that there was no fixation of genetic changes. Within thermotolerant populations, there was phenotypic variation in colony morphotype, with some populations showing morphotype changes associated with enhanced virulence. The model demonstrates a potential method to experimentally evolve C. neoformans under controlled conditions, providing a framework for long-term pathogen evolution studies.

37 – Determining Sex Differences in Spore-Mediated Cryptococcosis Using a Mouse Model

Sehrish Afsheen

Nicolas Pereira, Samantha Heather Ann McKechnie and Christina M. Hull

Department of Biomolecular Chemistry, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin, USA.

Human cryptococcosis exhibits a well-established sex bias, with males experiencing higher disease incidence and poorer clinical outcomes than females. The underlying bases for these differences (i.e., biological, cultural, social, etc.) are poorly understood. Although extensive research has been conducted on the virulence mechanisms of Cryptococcus, the effects of sex and sex hormone differences on infection outcomes remain largely unexplored. To evaluate a potential role for biological sex in cryptococcal disease, we infected both male and female mice (as determined by anogenital distance at weaning) with Cryptococcus and evaluated disease progression and outcomes. Male and female C57/BL6 and BALB/c mice were infected intranasally with either spores or yeast (B3501 and B3502 strains) and assessed for days of survival after infection and organ fungal burdens at end point. In both mouse backgrounds, male mice succumbed to disease faster than female mice (C57/BL6, $p = 0.0359$ and BALB/c, $p < 0.0001$). Fungal burdens in the brain, lung, kidney, and spleen did not differ between sexes at end point. From these data, we hypothesize that dissemination from the lung occurs earlier in males, leading to lethal fungal organ burdens earlier than in females. Future experiments will 1) determine timelines of lung dissemination in males vs. females, 2) identify any differences in lung immune cell interactions with spores between males and females, and 3) determine the roles of sex hormones in controlling time-to-disease. This study highlights a potential need for sex-specific therapeutic considerations when treating cryptococcosis.

38 – Immune Mechanism of Programmed Cell Death of Macrophage in Prognosis of Non-HIV Cryptococcal Meningitis

Ying-Kui Jiang

Ying-Kui Jiang, Jia-Hui Cheng, Ling-Hong Zhou, Jun-Tian Huang, Wen-Jia Qiu, Hua-Zhen Zhao, Rong-Sheng Zhu, Hui Tang, Si-Qi Zhu, Li-Ping Zhu

Department of Infectious Diseases, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, National Medical Centre for Infectious Diseases, Huashan Hospital, Fudan University, Shanghai, China

Background and aims: Excessive host immune responses may contribute to poor prognosis in non-HIV cryptococcal meningitis (CM). Programmed cell death (PCD), including pyroptosis, apoptosis, and necroptosis, plays a pivotal role in host-pathogen interactions; however, its involvement in non-HIV CM is poorly understood. This study aimed to characterize PCD profiles in non-HIV CM and to explore the macrophage PCD-related immune mechanisms associated with disease prognosis. Methods: PCD-related gene expressions were assessed in monocyte-derived macrophages (MDMs) and correlated with baseline cerebrospinal fluid cytokines, fungal burden and disease severity. An in vitro RAW264.7 macrophage model infected with opsonized Cryptococcus neoformans H99 was established to investigate fungal load-dependent cytotoxicity and to explore the phenotype and the potential signaling pathways of PCD using RNA-sequencing, quantitative PCR, western blotting and targeted pathway inhibition. Results: Patients with higher fungal loads showed a tendency toward elevated expression of pyroptosis and necroptosis markers in MDMs. Notably, the necroptosis marker RIPK3 was significantly elevated in severe cases. In vitro, opsonized Cryptococcus was phagocytized by RAW264.7 cells, leading to activation of pyroptosis and necroptosis, concomitant suppression of apoptotic executioners, and upregulation of thioredoxin-interacting protein (TXNIP) in a time or dose-dependent manner at both transcription and translation levels. RNA-sequencing also revealed marked transcriptional reprogramming characterized by early activation of inflammasome-related genes (NLRP3 and IL-1β). Pharmacological inhibition experiments indicated that macrophage pyroptosis was supposed to be mediated through a TLR4-TXNIP-NLRP3 inflammasome axis. Conclusions: High fungal burden in CM triggers inflammatory PCD dominated by TXNIP-mediated, NLRP3-dependent pyroptosis.

39 – The novel carbohydrate modifying enzyme Cel1 is required for Cryptococcus neoformans cell wall remodeling in response to host environmental stress.

Corinna Probst

Wan Y2, Hallas-Møller M3, Johansen K3, Dupree P2 and Alspaugh JA1

Duke University, School of Medicine Department of Medicine, Durham USA

The cell wall provides support and integrity to the fungal cell. As the interface with the external environment, it is also an important structure for stress adaptation. Accordingly, rapid changes within the carbohydrate composition and architecture occur in response to environmental stresses. Lytic polysaccharide monooxygenases (LPMOs) are copper-enzymes, secreted by microbes to assist in the first steps of remodeling and degradation of complex carbohydrates. While recent studies have established an important role for LPMOs in plant pathogenic microbes, studies about the role of LPMOs in human pathogenic microbes are scarce and limited to bacteria. The human fungal pathogen Cryptococcus neoformans (Cn) encodes for 4 potential LPMOs. Initial phenotypic analysis of loss-of-function mutant strains identified the CEL1 gene to be required for growth at mammalian host temperature and pH. In line with this finding, the cel1 mutant strain is avirulent in a murine model of infection. In contrast to other known LPMOs which are secreted into the extracellular environment and thereby targeting exogenous carbohydrates, CnCel1 is retained within the cryptococcal cell wall, suggesting a role in promoting intrinsic fungal cell wall remodeling. To further elucidate CnCel1 function we performed a combination of solid-state NMR, carbohydrate and microscopic analysis. These analyses suggest a novel role for the Cel1 enzyme in modulating cell wall chitosan distribution and deposition, a key cell wall carbohydrate required for stress adaptation during infection. Taken together, our recent work has demonstrated a novel role for an LPMO-family enzyme for fungal cell wall remodeling and stress adaptation during pathogenesis.

40 – Investigating novel lectibodies against the capsule of human fungal pathogen Cryptococcus neoformans

Christopher Lee

Christopher W. J. Lee1, Jie Jiao2, François Le Mauff3,7, Michael Lehky4, Elise Bedford1, Xianya Qu1, Cassie Zhong1, Stefan Mereiter5, Jack Paransky1, Sebastian Dawo2, Donald Sheppard3,8, Josef Penninger2,4,5,6, James Kronstad1

Michael Smith Laboratories

The capsule of Cryptococcus neoformans remains the most prominent virulence factor responsible for evasion of the host immune system. Unfortunately, no preventive or curative treatments have been successful in targeting the capsule. Novel antibody-lectin hybrid molecules, termed lectibodies, have been shown to mediate immune clearance of cancer cells and virus-infected cells thanks to the recognition of aberrant glycosylation patterns presented by the cells. We therefore hypothesize that lectibodies targeting the fungal capsule could represent a new therapeutic avenue to alleviate patient suffering from cryptococcal infections. We conducted a screen of 66 purified human recombinant lectibodies against encapsulated C. neoformans cells and identified four candidate lectibodies binding to the outer capsule in a calcium dependent manner. Specificity testing of the four hits revealed a preferential recognition of C. neoformans serotype A. However, no impact on cryptococcal growt h or capsule elaboration was observed in culture. The ability to facilitate phagocytosis by host bone marrow derived macrophages was investigated in a tissue culture system and we found that phagocytosis of fungal cells was significantly increased upon opsonization with the lectibodies. These results were consistently observed with human THP-1 monocyte derived macrophages, as well as BV-2 murine microglial cells. These results suggest that novel lectibodies may play a critical role in the host recognition of C. neoformans during infection, paving the way for exploiting these recognitions to develop innovative immunotherapy options.

41 – Invasive cryptococcosis with Klebsiella pneumoniae co-infection associated with refractory disease and high mortality: A case series

Dora Edith Corzo Leon

Eduardo Perez-Alba, Patricia Rodriguez-Zulueta, Eleni Didon, Matheus Araujo, Elizabeth Ballou

MRC Centre for Medical Mycology

Cryptococcus neoformans is the leading cause of fungal meningitis worldwide. Standard induction therapy combines amphotericin B with a second antifungal agent; however, treatment failure occurs due to drug resistance, inadequate dosing, monotherapy, or host immune dysfunction, leading to relapsing or refractory disease with high mortality. We report four cases of invasive cryptococcosis with concurrent Klebsiella pneumoniae bloodstream infection. All patients developed refractory disseminated cryptococcal disease despite initial combination antifungal therapy. Two of four patients (50%) died despite aggressive antifungal and antibiotic treatment. C. neoformans and K. pneumoniae isolates were recovered from three cases. We performed comprehensive antifungal and antimicrobial susceptibility testing and whole genome sequencing to characterize resistance patterns and genetic signatures. Clinical presentations, phenotypic resistance profiles, and genomic analysis revealed antifungal resistance signatures in all characterized strains. The concurrent bacterial-fungal co-infection was associated with poor outcomes despite appropriate combination therapy. This case series highlights the clinical challenge of cryptococcal-Klebsiella co-infection and suggests that concurrent bloodstream infections may contribute to antifungal treatment failure and mortality. Further investigation is needed to determine whether bacterial co-infection influences cryptococcal virulence or treatment response.

42 – Investigating interactions between macrophage ridges and Cryptococcus neoformans

Rebecca Hodges

Simon A Johnston 1, Rhoda Hawkins, Jaime Canedo 1

School of Medicine and Population Health, Florey institute, Bateson Centre - The University of Sheffield

Cryptococcus neoformans (Cn) is an opportunistic pathogen causative of serious infection of the central nervous system and the primary causative agent of cryptococcal meningitis (CM), a lethal infection predominantly affecting people living with HIV. We hypothesise that increasing phagocytosis of Cn by macrophages is a potential adjunctive therapy. We and others have extensively characterised the parasitism of macrophages by Cn, including in-macrophage growth. However, phagocytosis of Cn by macrophages is essential for surviving infection in both protective and susceptible animal models of cryptococcosis. This in vitro/vivo disparity was explained by us directly measuring macrophage parasitism in vivo in zebrafish demonstrating reduced overall fungal burden despite in-macrophage growth, even in a susceptible model. In agreement with this, human susceptibility to CM results from loss of in macrophages that can phagocytose cryptococci. In CM, the levels of macrophage-targeting in phagocytosis are a positive predictor of survival (independently of anti-fungal treatment). Thus, experimental and clinical data support our hypothesis that phagocytosis is key to surviving life-threatening infection and may be a therapeutic target for CM. Therefore, we have sought new ways to increase the phagocytosis of Cn and have discovered sub-micron actin-based ridges on the macrophage surface which are used to bind and phagocytose cryptococci. However, on exposure to Cn, macrophage ridge formation is attenuated, we aim to elucidate the mechanism by which Cn modulates this interaction.

43 – Simulating actin-driven phagocytosis of Cryptococcus neoformans

Dionn Hargreaves

James Bradford, Rhoda Hawkins, Simon A Johnston

University of Sheffield

The actin cytoskeleton is a central component of almost all animal and plant cells and plays a critical role in a wide range of biological processes. In humans, these include normal organ development, cancer, and immune defence. Within the immune system, actin dynamics are essential for phagocytosis, where coordinated cytoskeletal reorganisation drives the formation and progression of the phagocytic cup. This process is particularly important for the clearance of fungal pathogens such as Cryptococcus neoformans, which enters the body via the lungs and can cause life-threatening cryptococcal meningitis in immunocompromised patients. Notably, C. neoformans exhibits a range of sizes and morphologies that challenge effective phagocytic uptake. The overall function of the actin cytoskeleton arises from the collective behaviour of actin filaments and associated regulatory proteins. Among these, the Arp2/3 complex plays a key role by nucleating branched actin networks that are thought to be crucial for efficient target engulfment. We present a computational modelling framework that describes the kinetics and dynamics of the actin cytoskeleton using Monte CarloBrownian dynamics simulations. The model combines stochastic Monte Carlo approaches with Brownian dynamics to enable large-scale network remodelling to emerge from small-scale interactions. This work demonstrates how stochastic models of the actin cytoskeleton can be used to investigate immune responses to fungal infection, providing mechanistic insight into hostpathogen interactions during cryptococcal phagocytosis. This complements experimental studies by supporting hypothesis generation and potentially reducing reliance on costly and technically challenging in vitro and in vivo models.

44 – Titan cell formation is essential for C. neoformans latency

Rachel Berg-Murante

Kirsten Nielsen

Virginia Tech

Virtually everyone is exposed to Cryptococcus in childhood by inhalation of fungal cells, with an estimated 95% of adults harboring a persistent subclinical Cryptococcus infection in their lungs. This latent infection reactivates to cause disseminated disease in immunocompromised people yet little is known about how this latency arises. Our lab developed a mouse model of latent C. neoformans infection to define both host and pathogen factors that influence latency. Titan cells are an important virulence factor produced during C. neoformans pulmonary infection. Previous studies showed that deletion of the G-protein coupled receptors gpr4 and gpr5 significantly reduced titan cell formation from 30% to <5% and resulted in attenuated disease in the KN99 acute infection model. To determine the role of titan cells during latent infection, we generated a gpr4 gpr5 mutant in the latent isolate UgCl223. During latent infection, the pulmonary UgCl223 cell population consisted of 70% titan cells whereas the gpr4gpr5 mutant had <20% titan cells at 14 days post infection (dpi). Surprisingly, no titan cells were detected in the gpr4gpr5 mutant by 30 dpi while the wildtype strain retained its high titan cell production. Fungal burden was significantly lower in the gpr4gpr5 mutant at 30 dpi, with clearance of the gpr4gpr5 mutant by 90 dpi. These results indicate titan cell formation is critical for essential for persistence of latent infections and could serve as an effective target for new treatment strategies.

45 – Analysis of Cryptococcus spp. Extracellular Vesicles and Their Role in the Modulation of Gene Expression in Endothelial Cells

SUELEN ANDREIA ROSSI

Sulen Andreia Rossi1; Amanda Ferreira F. Kato1, Marianna Dallastella1, Gabrieli M. Huff1; Kayo Schemiko Almeida1; Tatiana P. Coutinho1; Gabriel P. L. da Silva1; Ricardo Amorim1; Lysangela Ronalte Alves1.

Carlos Chagas Institute, FIOCRUZ, PR, Brazil.

Cryptococcus spp. are encapsulated fungi responsible for cryptococcosis, an important systemic mycosis that mainly affects immunocompromised individuals. One of the important aspects of the virulence of these fungi is the production of extracellular vesicles (EVs), which are capable of transporting virulence factors to the extracellular environment and may modulate the response of recipient cells. In this study, we investigated the differences between EVs produced by two Cryptococcus species: C. neoformans (strain H99), which mainly affects immunocompromised patients, and C. deuterogattii (strain R265), which is also capable of infecting immunocompetent individuals. For this purpose, analyses of RNA and protein contents of EVs from both strains were performed, as well as the response of HUVEC cells treated with these EVs through transcriptomic analysis to evaluate modulation of gene expression. Proteomic analysis demonstrated a variety of shared proteins (112), while 82 proteins were found exclusively in C. neoformans EVs and 38 exclusively in C. deuterogattii EVs. Transcriptomic analysis of HUVEC cells revealed only 19 shared transcripts, whereas 41 transcripts were differentially expressed exclusively in cells treated with C. deuterogattii EVs and 211 in cells treated with C. neoformans EVs. In the presence of EVs, modulation of gene expression in HUVEC cells was observed; in both groups, there was increased expression of transcripts related to cell communication and response to stimuli. In conclusion, our study demonstrates that Cryptococcus extracellular vesicles can modulate gene expression in endothelial cells, reinforcing their importance in cellular communication during fungal infection.

46 – Cryptococcus neoformans serotype A virulence and pathogenicity are capsular glucuronoxylomannan (GXM) motif composition dependent

Samuel Rodrigues Dos Santos Junior

Piotr R Stempinski 1, Marcelo Valdemir de Araujo 2, Kevin Rojas 1, Gracen R Gerbig 1, Livia C Liporagi Lopes 1 3, Daniel F Q Smith 1, Maggie P Wear 1, Arturo Casadevall 1

W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA.

The Cryptococcus neoformans extracellular polysaccharide (EPS) is essential for virulence and host immunomodulation. Here, we analyzed the host pathogen interaction of four C. neoformans strains with different polysaccharide structures: two multi-motif (H99 and KN99) and two single-motif (Mu-1 and ATCC-24064 (24064)). We compared a variety of virulence and pathogenic factors, like capsule and cell body size, melanization, urease, and laccase production. Mice were also infected by the intranasal (IN) or intravenous (IV) route. Survival, fungal load, cell lung population, cytokines, and histology were analyzed. No differences in cell size or capsule size were found between the four strains. However, polysaccharide sizes were highly variable, and no significant differences were observed for all other virulence factors. Polysaccharides from single and multi-motif strains elicited different effects on Nitrite and reactive oxygen species production in macrophage-like cells and dendritic-like cells in vitro. There were differences in lung neutrophil infiltration between the analyzed strains, and a higher presence of activated macrophages and CD4+ lymphocytes for the Mu-1 or 24064 strains. Cytokines differed from the IN and IV infections. Lung histology showed increased inflammatory cell presence after infection with Mu-1 or 24064 strains, with no cryptococcal cells observed in the lung parenchyma or alveoli, whereas the opposite pattern was seen after challenges with H99 or KN99. These results suggest that variation in polysaccharide composition and structure can translate into differences in virulence and pathogenicity.

47 – Cryptococcus modulates host immune tolerance through STING-dependent inhibition of PFKFB3

Linghong Zhou

Juntian Huang, Yingkui Jiang, Liping Zhu

Huashan Hospital, Fudan University

Background and aims: Immune tolerance is a critical determinant in the pathogenesis of non-HIV-associated cryptococcal meningitis (CM). While stimulator of interferon genes (STING) is a known negative regulator of antifungal immunity, its mechanistic role in mediating immune tolerance remains unclear. This study aims to elucidate how STING interacts with glycolysis-related pathways to mediate immunometabolic suppression in non-HIV CM. Methods: Peripheral blood monocytes were collected from non-HIV CM patients, pulmonary cryptococcosis (PC) patients, and healthy controls (HC). Multi-platform analyses were conducted, including cytokine profiling, STING activation assessment, and glycolytic flux quantification. In vitro immune-tolerant models and murine cryptococcal infection models were established, utilising STING genetic knockout and glycolytic rescue experiments. Molecular docking and Co-IP were employed to validate interactions between STING and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3). Results: CM monocytes displayed significantly impaired TNF-/IL-6 secretion and reduced glycolytic intermediates, alongside elevated STING expression, relative to PC and HC cohorts. Notably, persistent glucuronoxylomannan (GXM) exposure recapitulated this tolerogenic phenotype in vitro. Pharmacological antagonism or genetic ablation of STING effectively rescued cytokine production. In vivo, STING-/- mice demonstrated significantly improved survival and reduced fungal burden. Mechanistically, molecular docking and Co-IP verified a direct high-affinity interaction between STING and PFKFB3. Consequently, STING deficiency reversed GXM-mediated PFKFB3 suppression. Conclusions: Cryptococcal infection upregulates STING, which directly binds and inhibits PFKFB3, suppressing glycolysis and inducing immune tolerance. Targeting the STING-PFKFB3 axis restores immunometabolic function and enhances antifungal responses. These findings establish STING as a master regulator of immunometabolic reprogramming and provide a novel therapeutic strategy.

48 – Genetic and pathogenic diversity of Cryptococococcus gattii

Qinxi Ma

Grace J. Paul1, Nicolas Helmstetter1, Robin C. May2, Matthew C. Fisher3, Rhys A. Farrer1

MRC Centre for Medical Mycology, University of Exeter

Cryptococcosis is a severe fungal disease caused by Cryptococcus neoformans and its sister species C. gattii. Advances in whole-genome sequencing and population genomics have revealed six genetically distinct C. gattii lineages (VGIVGVI). C. gattii displays massive phenotypic diversity. We performed genomic analysis on 46 C. gattii strains, including 25 clinical isolates, 15 environmental isolates, 3 veterinary isolates and 3 of unknown origin gathered from 13 countries across six continents. Phylogenetic analysis of the 46 isolates revealed a clear separation into the 6 distinct lineages. We evaluated virulence of these C. gattii isolates using the insect model Galleria mellonella. Only 6 out of the 46 isolates caused death in all larvae after 10 days post infection, whereas 5 caused less than 50% killing after 10 days post infection. Among the six lineages, VGI and VGIII were the least virulent. We also observed substantial intra-lineage variations, which likely reflec ts intra-lineage genetic diversity. Surprisingly, we observed that in the insect model, VGV isolates showed on average ~80% mortality, which may be due to the lack of adaptive immunity. Survival of larvae did not differ significantly between infections with environmental and clinical isolates (12.5% and 15%, respectively). Given that C.gattii is commonly inhaled by the host from the environmental sources, it is unsurprising that no notable differences were found between environmental and clinical isolates.These findings underscore the substantial genetic and phenotypic diversity within the C. gattii complex and highlight the value of integrating genomic and infection-model data to elucidate the evolutionary and pathogenic mechanisms.

49 – In vivo targeting of Hsp90 in murine models using positron emission tomography, magnetic resonance imaging and computed tomography.

Uwe Himmelreich

Romy Cools, Luigi Musetta, Koen Vermeulen, Dries Maes, Lara Roosen, Greetje Vande Velde, Katrien Lagrou, Guy Bormans

KU Leuven, Department of Imaging and Pathology, Faculty of Medicine, Leuven, Belgium

Introduction: Heat shock proteins (Hsp) contribute to one of Cryptococcus neoformans main virulence factors, its ability to grow at elevated temperature. Targeting Hsp90 has emerged as a potential approach to combat cryptococcosis. Using positron emission tomography (PET), tracers to target Hsp90 serves both diagnostic but also therapeutic purposes. Methods: Binding specificity of [11C]HSP990 to C.neoformans was assessed through pre-incubation with HSP990. In vivo PET/MRI and PET/CT studies of [11C]HSP990 were conducted in Balb/C mice following intratracheal (10^5 H99) or intracranially injection (10^4 H99). PBS and LPS injections acted as negative and positive controls. PET studies were performed under baseline conditions and after pre-treatment with HSP990 (5 mg/kg, ip) to evaluate in vivo Hsp90 binding specificity. Post-mortem in vitro Hsp90-specific binding of [11C] HSP990 was evaluated using autoradiography. Pathogenesis and lesion formation of cerebral cryptococcosis was confirmed by MRI, bioluminescence and CT imaging. Results: [11C]HSP990 binding to cryptococci was higher at 37ºC compared to 30ºC and RT. Tracer binding was confirmed to be Hsp90-specific, as significant reductions were observed after blocking with Hsp90 inhibitors. In vivo PET imaging of infected mouse brains demonstrated Hsp90-specific [11C] HSP990 uptake, with isointensity between lesions and healthy brain. In contrast, in vivo PET imaging of infected mouse lungs showed significantly higher Hsp90-specific tracer retention in infected lungs compared to healthy tissue and compared to LPS-positive and PBS-negative controls. Conclusions: [11C]HSP990 is a suitable tracer for in vivo diagnosis and treatment follow-up of cryptococcosis with excellent contrast in particular in infected lungs and good BBB penetration.

50 – Affordable PEGylated liposomal amphotericin B enables effective and safe oral treatment of experimental murine cryptococcosis

Ludmila Gouveia-Eufrasio

Victor Augusto Teixeira Leocádio 1, Guilherme Santos Ramos 2, Danielle Leticia Silva 1, Barbara Maria de Amorin Santos 1, Helane Lúcia Oliveira de Morais 2, Pedro Henrique Reis da Silva 3, Julia Facci Colangelo 3, Christian Fernandes 3, Daniele da Glória de Souza 1, Nalu Teixeira Aguiar Peres 1, Frédéric Frézard 2, Daniel Assis Santos 1,4

Departament of Microbiology, Universidade Federal de Minas Gerais, Belo Horizonte, 31270-901, Brazil

Cryptococcosis is a life-threatening fungal infection that predominantly affects immunocompromised individuals and remains a major global health challenge. Amphotericin B (AmB) is a central pillar of therapy; however, its clinical use is limited by nephrotoxicity, high cost, and the need for intravenous administration. In this study, we developed an affordable PEGylated liposomal AmB formulation (LAmB) using low-cost lipids and a simple, scalable ethanol injection method, and evaluated its oral efficacy and safety in a murine model of Cryptococcus neoformans H99 infection. The formulation produced uniform nanoparticles with a mean diameter of approximately 110 nm and an encapsulation efficiency exceeding 95%. Oral administration of LAmB significantly improved survival, achieving complete protection of infected animals, and markedly reduced fungal burden in the lungs and brain when compared with untreated mice and animals receiving conventional AmB deoxycholate. Histopatholo gical analysis demonstrated substantial preservation of pulmonary architecture in LAmB-treated mice, accompanied by a pronounced reduction in pulmonary inflammation. Consistently, LAmB treatment significantly decreased CXCL10 and IL-6 levels in lung tissue and bronchoalveolar lavage fluid. Toxicological evaluation revealed only mild renal alterations and no evidence of hepatotoxicity, consistent with the known safety profile of AmB formulations. Overall, these findings demonstrate that orally administered PEGylated liposomal AmB combines potent antifungal efficacy with reduced inflammation and acceptable safety. Its oral bioavailability and low-cost composition support its potential as a promising and accessible therapeutic strategy for treating cryptococcosis, particularly in resource-limited settings.

51 – Experience of research nurses with oral Encochleated amphotericin B for treatment of Cryptococcal Meningitis in a resource-limited setting

Cynthia Ahimbisibwe

Richard Kwizera (1), Jane Frances Ndyetukira (1), Olivie Carolyne Namujju (1), Alisat Sadiq (1), Mucunguzi Atukunda (1), Caleb P Skipper (2), David R Boulware (2), David B. Meya (1,2)

Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda.

Background and aims Cryptococcal meningitis accounts for upto 19% of AIDS-related deaths. First-line therapy uses either seven-day induction with intravenous amphotericin B deoxycholate and oral flucytosine or single-dose liposomal amphotericin with oral flucytosine and fluconazole. Besides the commonly used intravenous formulations of amphotericin B, an oral nanocrystal amphotericin B (MAT2203) formulation is being evaluated for efficacy to treat invasive fungal infections. This new experimental oral formulation has not been used before. Herein, we aimed to describe our experiences with using oral cAmB in a resource-limited setting from the perspective of research nurses. Methods This was part of a sequential Phase I and then Phase II multi-site randomized controlled trial that aimed to investigate the efficacy of oral nanocrystal amphotericin B formulation among persons with cryptococcal meningitis. Results We screened 240 participants in the two trials and enrolled 168 of which 81 (48%) were women. We found oral amphotericin a better alternative to intravenous amphotericin B deoxycholate due to less toxicity, mostly limited to gastrointestinal-related toxicities only. We clinically observed no drug reactions like rigors, phlebitis, and less vomiting among patients on oral amphotericin as compared to those on intravenous amphotericin B deoxycholate. Subjectively, meningitis symptoms of patients on oral amphotericin seemed to overall clinically improve more rapidly compared to those receiving intravenous amphotericin B deoxycholate. Few adverse events were observed. Conclusions In conclusion, oral amphotericin B was generally safe and well tolerated. However, it requires some training for the nurse, patient and caretakers for better administration, adherence and treatment outcomes.

52 – Targeting the electron transport chain as a strategy for novel antifungal drugs against Cryptococcus neoformans

Dmytro Prasolov

Prof. Campbell Gourlay, Dr. Elizabeth Edrich, Dr David Beal, Amy Powell

University of Kent

Cryptococcus neoformans remains a major cause of fungal meningitis, particularly in immunocompromised patients, highlighting the urgent need for new antifungal therapies with activity in host-relevant environments. Here, we demonstrate that our drug exhibits potent anticryptococcal activity through disruption of fungal bioenergetics and virulence-associated phenotypes. Treatment with the compound significantly reduced cellular respiration, ATP levels, and viability of C. neoformans. Importantly, its antifungal activity was retained in both nutrient-rich YPD medium and artificial cerebrospinal fluid (aCSF), supporting its potential utility for the treatment of cryptococcal meningitis. In addition to impairing growth and survival, the drug inhibited key pathogenic traits of C. neoformans. Under capsule-inducing conditions, it prevented capsule development, while under titan cell-inducing conditions in vitro, it significantly impaired titan cell formation. These findings suggest that the compound targets not only fungal survival but also mechanisms linked to immune evasion and disease progression. In vivo, the drug showed no detectable toxicity in the Galleria mellonella model and significantly reduced mortality in infected larvae, indicating therapeutic efficacy. Furthermore, the compound was non-toxic to red blood cells and remained stable in blood, supporting a favorable preliminary safety and stability profile. Collectively, these results identify our drug as a promising antifungal candidate with activity against C. neoformans in both standard and host-relevant conditions.

53 – Development of a physiologically relevant high-throughput screening assay for Cryptococcus neoformans drug discovery

Juliana da Silva Pacheco

Marta Garcia Sanchez* & Manu De Rycker

Drug Discovery Unit, School of Life Sciences, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dow Street, Dundee, DD1 5EH.

Cryptococcus neoformans is the main cause of fungal meningitis worldwide. Current antifungal therapies for cryptococcosis are limited by toxicity, accessibility and treatment efficacy, underscoring the need for novel antifungal compounds. To support drug discovery efforts, we developed and validated a physiologically-relevant 384-well high-throughput screening assay in Human Plasma-Like Medium (HPLM), a culture medium that more closely mimics the host environment and promotes cryptococcal morphotypes associated with infection.

The assay was systematically optimised to ensure robustness and reproducibility. Key parameters evaluated included seeding density, assay sensitivity and limit of detection, and tolerance to dimethyl sulfoxide (DMSO), the solvent commonly used for compound libraries. In parallel, we established a complementary assay format to assess compound fungicidal activity, enabling discrimination between growth inhibition and cell killing.

During assay development, several technical challenges were identified, including non-uniform growth patterns across screening plates that affected signal consistency. These edge effects were minimised using gas-permeable membranes, resulting in improved assay performance and plate-to-plate reproducibility.

The final assay provides a platform for high-throughput primary screening against C. neoformans, which will enable identification and prioritisation of antifungal compounds with a high probability of translational relevance.

54 – Wave of hope: Marine bacteria produce compounds active against Cryptococcus

Teresa Pissarro

Francisco Xavier Nascimento 1, Maria Rita Ventura 1 and Catarina Pimentel

Instituto de Tecnologia Qumica e Biolgica Antnio Xavier, NOVA University of Lisbon - ITQB NOVA, Oeiras, Portugal

Cryptococcosis is among the most concerning fungal diseases caused by opportunistic yeasts and is associated with high levels of mortality and morbidity, partly due to the limited availability of effective drugs, as recently highlighted by the WHO. In this context, the microbiota of marine organisms has attracted great interest as a sustainable and low-cost source for the discovery and production of new antifungals with superior efficacy.

Methods: A bacterial library isolated from the microbiota of two marine microalgae was screened for antifungal activity using agar diffusion assays. Genomes of active bacterial strains were analyzed for the presence of relevant biosynthetic gene clusters (BGCs). Promising compounds were chemically synthetized and tested for their antifungal activity using standard broth-dilution assays. Intracellular pH was measured using a strain expressing a GFP-pH-sensitive protein. Cryptococcus neoformans capsule size was observed through contrast microscopy and measured with ImageJ.

Results: Among the 39 bacterial isolates with antifungal activity against yeast, the most active strain contained a BGC for pentabromopseudilin (PBP). Chemically synthetized PBP was tested for antifungal activity against fungi listed in the WHO critical priority group. PBP decreased the intracellular pH, acting as a protonophore. PBP showed a very strong activity against Cryptococcus neoformans, with treated cells showing a 60% reduction in capsule size.

Conclusions: Marine bacteria secrete compounds with antifungal potential, such as PBP, which is highly active against Cryptococcus neoformans.

55 – Multi-omics analysis of extracellular vesicles secreted by Cryptococcus neoformans and their role in modulating the chronic inflammatory process

Anamelia Lorenzetti Bocca

GABRIÉLLY B. TRINDADE 1,2; RAFAELA L.L SOUZA 1,2; JULIANA C. SILVA 1,2; JOÃO SANTANA DA SILVA 2, ANAMéLIA L. BOCCA1,2,3

Bi-Institutional Translational Medicine, Oswaldo Cruz Foundation, São Paulo, Brazil and Center of Biotechnology, University of Brasília, Brasília, Brazil.

Cryptococcus neoformans is a major opportunistic fungal pathogen and a leading cause of life-threatening meningoencephalitis in immunocompromised individuals, particularly following primary pulmonary infection. Protective immunity against cryptococcosis is predominantly mediated by CD4+ T celldriven Th1 and Th17 responses, orchestrated by dendritic cells (DCs), which promote classical (M1) macrophage activation and effective fungal clearance. The efficiency of this process critically depends on the pulmonary microenvironment, the expression of co-stimulatory molecules, and the quality of antigen processing and presentation by DCs. Extracellular vesicles (EVs) released by fungi have emerged as important mediators of hostpathogen communication, acting as carriers of complex bioactive cargo that modulate immune cell activation, cytokine production, and inflammasome signaling, thereby influencing disease outcome. Although environmental and nutritional conditions are known to affect fungal physiology, how nutrient availability reshapes the molecular composition of C. neoformans EVs and their immunological functions remains poorly defined. In this project, we apply integrated multi-omics approaches, including proteomics, lipidomics, and metabolomics, to systematically characterize EVs produced by C. neoformans cultured under distinct nutrient availability conditions (100%, 50%, and 10%). We further assess how nutritionally driven alterations in EV cargo modulate DC maturation and activation in murine models of pulmonary infection and how these changes translate into differential local immune responses in the lung. To enhance translational relevance, we extend our analyses to primary human DCs obtained from HIV-infected individuals, with and without cryptococcal co-infection. Preliminary LCMS/MS-based metabolomic and lipidomic profiling revealed that EVs derived from nutrient-rich and intermediate growth conditions are markedly enriched in bioactive lipid species, including phosphatidylethanolamines, phosphatidylcholines, ergosterol, and sphingoid bases. This distinct lipid signature suggests that EVs generated under favorable nutritional environments have potential immunomodulatory and immunosuppressive capacities, which may contribute to the establishment of a permissive niche for fungal persistence within the host. Together, our findings support a model in which nutritional cues dynamically reprogram the molecular composition and immunological impact of C. neoformans EVs, with direct implications for host defense and disease progression.

SESSION 2

56 – Predictors of 90-Day Mortality Across Immune Groups in Adults with Cryptococcal Meningoencephalitis

Daniel Chastain

Bayard A. Taylor, Ameera Jamshad, David B. Cluck, Charlie Garcia, Andres F. Henao-Martinez

Clinical Associate Professor, University of Georgia College of Pharmacy

Background and Aims Mortality predictors in cryptococcal meningoencephalitis (CM) across HIV, solid organ transplant (SOT), and non-HIV, non-transplant (NHNT) adults are unclear. We compared clinical characteristics and 90-day mortality by immune status. Methods We identified adults (18 years) with CM in TriNetX (20032024) using ICD-10-CM codes or cerebrospinal fluid confirmation. Analyses were restricted to amphotericin Btreated patients. Patients were categorized as HIV, SOT, or NHNT. Clinical characteristics, immunosuppressive exposures, and outcomes were compared by 90-day mortality, with time-to-event analyses using KaplanMeier and Cox models. Results Among 766 patients, 26% had HIV, 22% had prior SOT, and 52% were NHNT. Ninety-day mortality was 19% overall (23% in NHNT, 20% in SOT, and 13% in HIV). In HIV-associated CM, non-survivors more often had hematologic malignancy (14% vs 2%) and liver disease (18% vs 3%). In SOT recipients, non-survivors had higher rates of hematologic malignancy (14% vs 3%), aplastic anemia (48% vs 10%), and ICU admission (66% vs 25%). Among NHNT patients, non-survivors were older (63 vs 56 years), more likely to have neoplastic disease (39% vs 23%), and require ICU care (55% vs 28%). Immunosuppressive exposures were similar across groups. NHNT status was associated with increased 90-day mortality (HR 1.98) in unadjusted analysis, but attenuated after adjustment for age, comorbidity, and glucocorticoids, with age remaining associated with death (HR 1.02/year). KaplanMeier curves demonstrated early, persistent separation by immune status. Conclusions Higher mortality in NHNT adults reflects host fragility rather than immune status, supporting severity-based risk stratification.

57 – Clinical Phenotypes and Immunosuppressive Medication Exposures in Pulmonary Versus Extrapulmonary Cryptococcosis Among Non-HIV, Non-Transplant Adults: A U.S. Claims Analysis

Daniel Chastain

Ameera Jamshad, Bayard A. Taylor, Guozheng Yang, Zhetao Chen, Xianyan Chen, David B. Cluck, Andrés F. Henao-Martinez

Clinical Associate Professor, University of Georgia College of Pharmacy

Background and Aims Cryptococcosis in non-HIV, non-transplant (NHNT) adults is increasingly recognized yet clinically heterogeneous. We characterized clinical features of NHNT cryptococcosis and evaluated associations between disease phenotype, comorbidities, immunosuppressive medication exposures, and outcomes. Methods NHNT adults (18years) with ICD-10-CM cryptococcosis codes in Merative MarketScan (20162023) were classified as having pulmonary or extrapulmonary disease. Immunosuppressant, systemic glucocorticoid, and antineoplastic (WHO ATC classification) exposures were assessed 180 days before diagnosis. Outcomes included ICU admission, duration of hospitalization, and discharge disposition. Results Among 722 NHNT adults, 52% had extrapulmonary cryptococcosis. Extrapulmonary disease occurred in younger patients with greater multimorbidity than pulmonary disease (81% vs 62%, $p<0.001$). Only 5% lacked comorbidities, compared with 12% of pulmonary cases. Autoimmune and immune-mediated diseases were more frequent in extrapulmonary disease (20% vs 16%), while chronic lung disease was less common (9% vs 21%). Immunosuppressant exposure was higher in extrapulmonary cases (26% vs 11%, $p<0.001$), whereas antineoplastic exposure did not differ (10% vs 13%). Glucocorticoid exposure was similar (57% vs 60%, $p=0.560$), predominantly prednisone. Cumulative prednisone-equivalent dose was similar between extrapulmonary and pulmonary cases (median 600 vs 230mg, $p=0.072$), but extrapulmonary cases had longer prednisone exposure (median 60 vs 27days, $p=0.025$). Extrapulmonary cryptococcosis was associated with higher ICU admission (43% vs 18%), longer hospitalization (median 16 vs 7days), and fe wer home-based discharges (59% vs 21%) (all $p<0.05$). Conclusions Extrapulmonary cryptococcosis in NHNT adults is associated with greater immunosuppressive medication exposure, multimorbidity, and healthcare utilization, illustrating the need for improved awareness of this evolving at-risk population.

58 – Advanced HIV disease complicated by cryptococcal meningitis, disseminated tuberculosis, severe drug-induced liver injury, and heart failure.

Sylvia Namanda

Derrick Kasozi1, Suzan Namombwe1, Olivie C Namuju1, Richard Kwizera1, Suzan Mulwana1, David Boulware3, David B Meya1,2,3.

Department of Research, Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda.

Background: Advanced HIV disease (AHD) remains a major cause of morbidity and mortality in sub-Saharan Africa, frequently complicated by overlapping opportunistic infections and treatment-related toxicities. Managing concurrent cryptococcal meningitis and tuberculosis in profoundly immunosuppressed patients is particularly challenging, with high risk of sepsis, drug toxicities, and death. Case Presentation: We report a 35-year-old female, one year postpartum, with a 20-year history of HIV infection who defaulted antiretroviral therapy for one year and presented to Mulago National Referral Hospital, Uganda, with fever, cough, chronic diarrhea, weight loss, genital ulcer herpes, and confusion. She was diagnosed with cryptococcal meningitis and disseminated tuberculosis, with profound immunosuppression (CD4 20 cells/L). She was initiated on amphotericin B-based induction therapy, flucytosine, high-dose fluconazole, and anti-tuberculosis treatment. Her hospital course was com plicated by acute respiratory deterioration secondary to severe pneumonia, septic shock, cardiogenic shock with new-onset dilated cardiomyopathy and heart failure with reduced ejection fraction (EF 35-40%), amphotericin-associated ototoxicity, and severe drug-induced liver injury (DILI). Hepatotoxic drugs, which included anti-TBs were temporarily withheld, then successfully reintroduced using a stepwise re-challenge DILI protocol with close biochemical monitoring. Heart failure was managed with guideline-directed therapy. Antiretroviral therapy was restarted after stabilization. The patient was discharged after 51 days and remained clinically stable and adherent to therapy at 18-week follow-up. Conclusion: This case highlights the extreme clinical complexity of AHD management in resource-limited settings and demonstrates that survival is achievable despite multiple life-threatening complications.

59 – Therapeutic potential and synergistic effects of antifungal and off target drugs against clinical isolates of Cryptococcus laurentii

Saba Sana

Isha Ajmal

Department of Microbiology, Dr. Ikram Ul Haq Institute of Industrial Biotechnology. Government College Uniersity Lahore

Cryptococcus laurentii is an emerging opportunistic yeast pathogen associated with unpredictable antifungal susceptibility and biofilm-mediated resistance. Aims:This study investigated the therapeutic potential and synergistic interactions of standard antifungals (fluconazole, amphotericin B) and off-target drugs against clinical isolates of C. laurentii. Methods: Three clinical isolates recovered from blood (n=2) and cerebrospinal fluid (n=1) were confirmed by phenotypic characteristics and PCR amplification of the 18S rRNA gene (~480 bp). Biofilm formation was screened using Congo red agar and quantified via crystal violet assay. Susceptibility testing employed CLSI-guided disc/well diffusion and broth microdilution methods for antifungals and 14 off-target drugs. Minimum biofilm inhibitory concentrations (MBICs) were determined, and synergistic effects were evaluated using checkerboard assays (fractional inhibitory concentration index, FICI) in planktonic cells and biofilms, supplemented by coverslip biofilm reduction assay. Results: Isolates showed moderate biofilm production (mean OD595= 0.32±0.008), fluconazole susceptibility (mean MIC = 0.77 g/mL), and amphotericin B resistance (mean MIC = 4.48 g/mL). Diclofenac sodium exhibited the strongest off-target activity (mean MIC = 1.32 g/mL). Checkerboard assays revealed synergy between fluconazole + amphotericin B (FICI = 0.5) and fluconazole + diclofenac sodium (FICI = 0.32) in planktonic cells, with additive effects against biofilms (FICI = 0.9). The fluconazole-diclofenac combination significantly disrupted biofilm matrix. Diclofenac sodium enhances fluconazole efficacy and disrupts biofilms, supporting drug repurposing as an adjuvant strategy for managing biofilm-associated C. laurentii infections. Conclusion: So off-target drugs can be a suitabable alternatives t

60 – Non-HIV Cryptococcal Disease: A Case Series from a Tertiary Hospital in Peru

Nicolas Antunez de Mayolo

Mauricio Orozco Gonzalez, Omayra Chincha

Universidad Peruana Cayetano Heredia

Background and Aims: Cryptococcal disease is a severe fungal infection mainly associated with HIV but also occurs in non-HIV patients. In Peru, HIV remains the main risk factor; however, early recognition in non-HIV patients is essential. We aimed to describe the clinical characteristics, treatment, and outcomes of non-HIV cryptococcal disease. Methods: Retrospective case series of patients with mycologically confirmed cryptococcosis and non-reactive HIV ELISA (20182024). Clinical, laboratory, treatment, and outcome data were collected, and CSF parameters were analyzed according to 30-day mortality. Results: Seventeen patients were included; 88% had cryptococcal meningitis. Mean age was 52 14 years, and 53% were male. Immunosuppression was present in 64.7%, mainly corticosteroid use (35.3%); 35.3% had no identified risk factors. Common symptoms were headache (76.5%), fever (70.5%), and altered mental status (57.9%). Median CSF values were leukocytes 55 cells/mm^3, proteins 131 mg/dL, glucose 36 mg/dL, and mean opening pressure 33 cmHO. Most patients (94.4%) received amphotericin B plus fluconazole. Thirty-day mortality was 41.1%. No significant CSF differences were found by mortality, but effect sizes were large for opening pressure (d = 0.84) and moderate for hypoglycorrhachia (d = 0.57). Conclusions: Non-HIV cryptococcal disease was often associated with corticosteroid use, and one-third of patients lacked immunosuppressive risk factors. Elevated opening pressure and low CSF glucose may be associated with increased mortality.

61 – Diagnostic Challenge of Opportunistic Infections in Advanced HIV Disease: A Case of Disseminated Cryptococcosis.

Olivie Carolyne Namuju

Derrick Kasozi1, Jonathan Mulungi1, Morris K. Rutakingirwa1, Mawanda Anatoli2, Samuel Okurut1, Robert Lukande2, David Boulware3, David Meya1,3,4, Nicol Melanie5

Infectious Diseases Institute - College of Health Sciences - Makerere University

Background: Cryptococcosis, a deadly opportunistic fungal infection, can present as disseminated disease, including Cryptococcal Meningitis (CM) in immunocompromised patients. However, disseminated disease may present with non-specific signs and symptoms, evading standard screening algorithms, including serum cryptococcal antigen (CrAg) testing, yielding false-negative results due to the prozone effect. Methods: We report a case of disseminated cryptococcosis with soft tissue and pulmonary involvement in advanced HIV disease (AHD) patients diagnosed at postmortem. Results: A 30-year-old female, newly diagnosed with HIV, not on ART with CD4 of 04 cells/L, presented with a 2-month history of easy fatiguability associated with chest pain, non-productive cough, and dyspnea. On general examination, she was sick-looking with respiratory distress, wasted, and moderate pallor, without focal neurological deficits. Laboratory investigations revealed Grade 4 thrombocytopenia ($32x10^9$/L) and liver transaminitis. Urine TB lipoarabinomannan and serum cryptococcal antigen were negative. The chest radiograph showed bilateral hilar adenopathy. She was managed presumptively for Pneumocystis jirovecii pneumonia with high-dose dapsone. Despite supportive care, she passed on after 10 days hospitalization. Upon autopsy, gross examination of the lungs revealed heavy, consolidated lungs and subarachnoid clouding of the meninges, showing pulmonary parenchymal disease and meningitis. Microscopic examination of Periodic Acid Schiff stained sections revealed extensive involvement by cryptococcosis of the brain, lungs, spleen, kidneys, adrenal glands, heart, and pancreas. Conclusion: The case highlights diagnostic limitations of serum CrAg screening tests in AHD with non-specific systemic illness. Disseminated cryptococcosis may remain clinically unrecognized until death. Strengthening diagnostic algorithms for opportunistic infections in AHD is crucial.

62 – Incidence, reasons, and outcomes of rehospitalization among adults with HIV-related cryptococcal meningitis in Uganda: a retrospective cohort study

Rhona CB Muyise

Samuel Jjunju (1,4), Biyue Dai (2), David R. Boulware (3), David B. Meya (1,3), Richard Kwizera (1)

Infectious Diseases Institute, College of Health Sciences, Makerere University, P.O. Box 22418, Kampala, Uganda.

Background and aims: While advances in Cryptococcal meningitis (CM) treatment have improved survival, post-discharge outcomes such as rehospitalisation remain poorly understood. We aimed to determine the incidence, predictors, causes, and outcomes of re-hospitalisation by 10 weeks among adults with HIV/CM. Methods: This was a retrospective cohort study analysing data from four clinical trials conducted between 2017 and 2024 at Mulago, Mbarara, and Kiruddu Hospitals in Uganda. Adults with HIV/CM discharged alive after initial hospitalisation were followed for 10 weeks. Results: Of 1055 patients enrolled in the four clinical trials, 787 were discharged alive. All participants were adults with a median age of 35 years (IQR=30,40). All participants had HIV with a median CD4 cell count of 21 cells/L (IQR=8,58). The majority were male (59%), and 45% were ART-experienced. 209/787 (26.6%) experienced either rehospitalisation or death within 10 weeks. Seventy-six died without rehospitalisation, 25 died after rehospitalisation, 108 were alive by 10 weeks, and 578 had no event. GCS, duration on ART, Haemoglobin, CD4, sodium, CSF opening pressure >200, CSF protein, and CSF WBC were significantly different across the four groups. The median time from initial discharge to rehospitalisation was 20 days (IQR= 10, 36). The major cause of rehospitalisation was CM complications. Only normal GCS was significantly associated with rehospitalisation. However, age, GCS, CD4, haemoglobin and sodium were significantly associated with death. Conclusions: Nearly 3 in every 10 adults with HIV/CM experience rehospitalisation or death within 10 weeks following discharge, with the major cause of rehospitalisation being CM complications.

63 – Baseline hematologic abnormalities and survival in HIV-associated cryptococcal meningitis: a prospective cohort study

Jane Gakuru

Samuel Okurut1, Biyue Dai2, Suzan Namombwe1, Timothy Mugabi1, Faizo Ssekindi1, Sarah Kimuda1, Sarah Maria Najjuka1, Enos Kigozi1, Ciara Featherly1, Mable Kabahubya1, Laura Joan Nsangi1, Morris K. Rutakingirwa1,4, Darlisha Williams3, Caleb Skipper3, Conrad Muzoora5, Samuel Jjunju5, Edwin Nuwagira5, Tessa Adzemovic6, Felix Bongomin1,7, David Meya1,3,4, David Boulware3, Mahsa Abaasi3.

Infectious Diseases Institute, Makerere University, Kampala, Uganda.

Background Cryptococcosis is the leading cause of meningitis among people living with HIV. Hematologic abnormalities, particularly cytopenia, are frequently observed at presentation; however, their prognostic significance remains poorly defined. We evaluated the association between baseline cytopenia and survival in HIV-associated cryptococcal meningitis. Methods We prospectively enrolled participants between 2013 and 2025 at three hospitals in Uganda. Cytopenia was defined according to the Division of AIDS toxicity grading criteria as grade >=2 abnormalities in >=2 hematologic cell lines (white blood cells, hemoglobin, or platelets) at baseline. We compared baseline clinical and laboratory characteristics between participants with and without cytopenia and evaluated the association between baseline cytopenia and 4-week mortality. Results Among 1,729 participants presenting with HIV-associated cryptococcal meningitis, the median age was 35 years (interquartile range [IQR], 3042); 60% (1,035/1,729) were male, and 51% (883/1,729) were not receiving antiretroviral therapy at presentation. At baseline, 9% (149/1,729) had cytopenia, 12% (n=214) had leukopenia, 25% (n=432) had anemia, and 10% (n=167) had thrombocytopenia. Participants with cytopenia had lower median CD4 cell counts (9 cells/L [IQR: 4-7] vs 23 cells/L [IQR: 8-59], p<0.001), and lower median intracranial pressures (180 mm Hg [IQR: 111-245] vs 240 mm Hg [IQR: 150-350], p<0.001). Presence of cytopenia was associated with higher hazard of death at 4-weeks (adjusted-Hazard Ratio: 1.90; 95%CI, 1.452.48). Conclusion Baseline cytopenia is independently associated with substantially increased early mortality. These findings identify cytopenia as a readily available prognostic marker that may help risk-stratify individuals at presentation and inform the need for intensified monitoring.

64 – Changing impact of Cryptococcus on HIV-associated meningitis in northern Uganda

Abigail Link

Mark Okwir, David Meya, Ocora Boniface, Ritah NakatoPaul Bohjanen

University of Rochester

BACKGROUND & AIMS In past decades, Cryptococcus was the leading cause of death due to meningitis in sub-Saharan Africa among people living with HIV (PLWH). With improvements in HIV and cryptococcal meningitis (CM) care, we sought to evaluate the current causes of death or neurological impairment in PLWH with meningitis in northern Uganda. METHODS We conducted a retrospective cohort study utilizing data from PLWH admitted to Lira Regional Referral Hospital with meningitis from 2021-2025. Collected data consisted of demographic information, meningitis etiologies, in-hospital mortality and neurological deficits at hospital discharge, including neurocognitive sequelae such as confusion (GCS <14), delirium, aphasia, and behavior changes. RESULTS Among 276 PLWH with confirmed meningitis, 32.2% (n=89) were diagnosed with CM, 28.6% had bacterial meningitis (BM), 17% had tuberculosis meningitis and 8% had viral meningitis (VM). Thus, most PLWH with meningitis had etiologies other than Cryptococcus. Mortality due to CM (32.6%) was lower than for TBM (51.1%) and BM (48.1%). Those with TBM had more neurocognitive dysfunction at the time of hospital discharge (27.7%), compared to CM (15.7%) and BM (15.2%). However, among patients who died in the hospital, 71.4% of those with CM had neurocognitive dysfunction, compared to 38.5% of those with TBM and 25.0% of those with BM. CONCLUSION Among PLWH in Lira, northern Uganda, meningitis due to etiologies other than Cryptococcus occurred more commonly, although CM remains a major cause of death and neurological impairment.

65 – Bkt4, a phylogenetically restricted factor, links metabolic adaptation to cell-cycle regulation

Krishna Bhat

Kaustuv Sanyal

Molecular Mycology Laboratory, Molecular Biology & Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, India

Cryptococcal meningitis is a life-threatening disease caused by the opportunistic fungal pathogen Cryptococcus neoformans, which can disseminate to the central nervous system by crossing the blood-brain barrier. The success of C. neoformans cells to cause fungal meningitis depends on its ability to adapt to nutrient-limited host environments such as the lung and cerebrospinal fluid as well as to evade host immunity. Here, we identify Bkt4, an uncharacterized basidiomycete-specific nuclear protein, as a critical regulator of adaptation to glucose deplete conditions in C. neoformans. Bkt4 was originally identified as an interactor of kinetochore proteins along with bridgin in a previous screen. bkt4 null mutants show severely impaired growth not only under glucose-deplete conditions but also in the presence of alternative carbon sources, including galactose, maltose, and raffinose, suggesting that Bkt4 coordinates metabolic adaptation with cell-cycle progression. Furthermore, bkt4Δ cells exhibit attenuated virulence in the Galleria mellonella infection model and fail to undergo polyploid titan cell formation under in vitro inducing conditions. Genetic interaction analyses reveal functional associations between Bkt4 and kinetochore regulators, supporting a role for Bkt4 in integrating nutrient sensing with cell-cycle progression. Taken together, our findings identify Bkt4 as a novel basidiomycete-specific regulator linking metabolic adaptation to cell-cycle regulation in C. neoformans.

66 – Challenges and missing links to optimize nursing care for persons with HIV-associated meningitis in a resource-constrained setting

Jane Frances Ndyetukira

Shifah Nabbaale (1), Alice Namudde (1), Cynthia Ahimbisibwe (1), Peruth Ayebare (1), Grace B. Menya (1), Fred Turya Musa (1), Olivie C Namuju (1), Alisat Sadiq (1), David R. Boulware (2), David B. Meya (1,2,3), Richard Kwizera (1)

Infectious Diseases Institute, College of Health Sciences, Makerere University, P.O. Box 22418, Kampala, Uganda.

Background and aims: Nursing care is a vital component of managing persons with HIV-associated meningitis. We sought to describe the challenges faced during the nursing care of these patients, highlighting the gaps and recommendations to optimize nursing care in a resource-constrained setting. Methods: We included and interviewed ten nurses as key informants who were routinely involved in providing care for persons with HIV-related meningitis during nine clinical studies conducted between 2010 and 2025. Results: The human resource and infrastructure limitations include understaffing, limited knowledge, insufficient supplies, lack of space for counselling, limited bed space in hospital wards, limited number of physiotherapists for rehabilitation, and poor patient tracking post-discharge. The clinical and treatment-related complications include delayed diagnosis/treatment, raised intracranial pressure, Immune Reconstitution Inflammatory Syndrome, relapse, poor infection prevention and control, pill burden and multiple drug side effects. The socio-economic limitations include limited patient education, lack of patients social support, myths/misconceptions about medical procedures, religious beliefs, HIV stigma and non-disclosure of HIV status, patient/caregiver challenges and poverty of patients. The major missing links include the need to recruit more nurses, enhance training programs for nurses, empower nurses to gain expanded skillsets, ensure resource availability, provide more bed space , improve infection prevention and control, promote community awareness on HIV-associated meningitis, reinforce adherence to treatment guidelines, training caretakers about medication management and patient hygiene, and mentor nurses in research. Conclusions: Patients with HIV-associated meningitis require extra nursing care, including community engagement. Addressing the missing links will help to improve treatment outcomes.

67 – Saprobic and pathogenic Cryptococcus species: uncovering hidden diversity across the genus.

Marcia C David Palma

Marco A. Coelho (1), Aleksey V. Kachalkin (2,3), Miroslav Kolařík (4), Benedetta Turchetti (5), José Paulo Sampaio (6), Michael J. Wingfield (7), Matthew C. Fisher (8), Andrey M. Yurkov (9), Joseph Heitman (1).

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, United States of America

Species within the genus Cryptococcus range from opportunistic human pathogens to environmentally associated saprobes, yet the genomic and ecological processes underlying this diversity remain poorly understood. Comparative analyses across pathogenic and nonpathogenic lineages are essential for identifying evolutionary transitions linked to pathogenicity and for refining species boundaries. Here, we analyzed seventeen Cryptococcus species representing divergent evolutionary lineages, including six newly identified taxa and lineages previously lacking formal species designation. We integrated phylogenomic inference, divergence metrics, chromosome-scale genome analyses, mating assays, and metabolic phenotyping to clarify species boundaries and patterns of genome evolution within the genus. We formally designated Cryptococcus hyracis as the VGV lineage of the C. gattii species complex and described five new nonpathogenic species recovered from environmental substrates such as fruit, soil, and bark beetle galleries, three of which formed a previously unrecognized clade within Cryptococcus. One of these species, Cryptococcus porticicola, showed a strong association with bark beetles across multiple strains sampled from distinct geographic locations. Genomic analyses of this species revealed intraspecific chromosomal polymorphism, signatures of recombination, and evidence of sexual reproduction. Across Cryptococcus, we observed extensive karyotypic diversity, including lineage-specific rearrangements and repeated reductions in chromosome number, potentially linked to centromere loss or inactivation. Phenotypic Biolog profiling revealed clade-specific substrate preferences suggestive of ecological specialization, while growth at 37ºC was restricted to clinically relevant taxa. Together, these findings expanded the recognized diversity of Cryptococcus and provided new insights into the genomic and ecological forces shaping speciation and pathogenic potential.

68 – From Essential Metabolism to Resistance: Transposable Elements and 5-FAA Escape in Cryptococcus

Adriana Orrego

Rob van Nues (1), Isaac Martinez-Ugalde (3), Kundan Kumar (1), Sueden Oliveira De Souza (1), Cei Abreu-Goodger (2), Elizabeth Bayne (1)

Institute of Cell Biology, School of Biological Sciences, The University of Edinburgh, Edinburgh, UK

Transposable elements (TEs) can rewire genomes and gene expression, helping pathogens adapt under stress. In Cryptococcus, TE activity has been linked to antifungal resistance through disruption of key genes. Our recent research focuses on how TE mobilisation can also drive resistance to 5-fluoroanthranilic acid (5-FAA), a tryptophan (TRP) pathway antimetabolite. This is an especially interesting system in Cryptococcus because, unlike in Saccharomyces cerevisiae, the TRP biosynthetic pathway is essential. Classic loss-of-function resistance is therefore less plausible, pointing instead to regulatory changes, gene-dosage effects, or structural variationroutes in which TEs could play a key role. To test this, we used RNA interference (RNAi) as a lever, since it is the primary TE-silencing pathway. We compared a wild-type background with an RNAi-deficient strain, which is expected to show elevated TE activity. Using mutation-rate experiments, we observed an increased frequency of spontaneous 5-FAA-resistant colonies in the RNAi mutant. To identify causal variants, we conducted whole-genome sequencing analysis on resistant and non-resistant isolates. Initial findings indicate that de novo TE insertions in both UTRs of TRP5, a core TRP-pathway gene, are common in resistant isolates, suggesting resistance may arise through altered expression rather than gene inactivation. We are now extending the analysis to catalogue and validate additional structural variants associated with resistance. Together, this work aims to define how uncontrolled TE mobilisation can generate resistance to a compound targeting an essential pathwayprecisely the type of pathway often prioritised in antifungal drug discoveryin a significant human pathogen.

69 – Molecular and Phenotypic Characterisation of Clinical Cryptococcus Isolates

Alejandra Lora Plaza

Alejandra Lora-Plaza 1,Alba Torres-Cano 1, Oscar Zaragoza 1,2

Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain.

The Cryptococcus neoformans/gattii species complexes can be subdivided into serotypes, molecular types and genetic lineages based on structural, molecular or genetic differences, respectively. Hybrid strains have been reported both within (A/D) and between complexes (B/D). Differences in species and genotypes can influence patient type and antifungal susceptibility. Therefore, defining the molecular epidemiology of cryptococcal species is essential. This study aimed to identify and characterise clinical isolates from hospitals across Spain deposited at the Mycology Reference Laboratory of the Institute of Health Carlos III since 2020. Molecular identification was performed by conventional PCR and Sanger sequencing of the intergenic spacer region (IGS); hybrids (A/D or B/D) were detected using real-time PCR with molecular beacon probes. To date, 48 strains have been analysed. 81.8% were identified as C. neoformans, 57% of which belonged to genotype 1a (57%), followed by genotype 1b (14%) and 1c (6%). 11.3% were C. deneoformans, and 6.8% as A/D hybrids. The remaining 5% of isolates were C. deuterogattii (66.7%) and C. gattii (33.3%). Interestingly, all C. deuterogattii isolates were from the same geographical region (southern Spain). Regarding the origin, most of them were isolated from cerebrospinal fluid (49%) followed by blood samples (25%). Twelve isolates showed high fluconazole MICs (32 mg/L). Preliminary phenotypic analyses revealed capsule variability among clinical isolates. Growth assays showed that some strains exhibited markedly slow growth at multiple temperatures, but no clear correlation with genotype thus far. These early findings provide initial insight into the phenotypic diversity of Cryptococcus species associated with virulence.

70 – Heterochromatin Variation and Phenotypic Heterogeneity in Cryptococcus neoformans

Vishnu Priya Krishnan

Rebecca Yeboah, Pin Tong, Robin C. Allshire

Institute of Cell Biology, University of Edinburgh

Cryptococcus neoformans is a major opportunistic fungal pathogen that poses a severe threat to immunocompromised individuals, particularly those living with HIV/AIDS. Current antifungal therapies remain limited, and the increasing emergence of drug resistance presents a significant challenge to effective treatment. Among resistance phenomena, heteroresistance which is defined by the presence of transient, unstable resistant subpopulations within an isogenic population has gained attention as a key contributor to treatment failure. While aneuploidy has been widely implicated in fluconazole heteroresistance, recent observations of non-aneuploid resistant isolates suggest that additional, less understood mechanisms may also play a role. Emerging evidence points toward epigenetic regulation as a potential driver of such transient resistance states. In particular, heterochromatin formation through histone modifications such as H3K9 and H3K27 methylation has been associated with reversible gene silencing and adaption to antifungal stress. Preliminary findings indicate that resistant non-aneuploid isolates can exhibit ectopic heterochromatin domains that are absent in reference strains and are lost upon removal of drug pressure, suggesting a role for epigenetically mediated, reversible resistance mechanisms. This study investigates how heterochromatin dynamics shape antifungal resistance and phenotypic variability in C. neoformans. By profiling a diverse panel of clinical and environmental isolates, this work explores whether variation in heterochromatin distribution correlates with differences in drug susceptibility and adaptive potential. Particular focus is placed on mapping histone methylation peaks and identifying genomic regions where heterochromatin may influence genes linked to stress response, virulence, and drug resistance.

71 – Genomic signatures of parasex among two genetically distinct hybrid groups of Cryptococcus neoformans.

Rahul Anand

Rahul Anand1, Qinxi Ma1, Diana Tamayo1, Nicholas Helmstetter1, Rhys A. Farrer1.

University of Exeter

Hybridisation is an important source of genetic and phenotypic diversity in fungi, yet the mechanisms shaping hybrid genomes in human pathogens remain unclear. Cryptococcus neoformans, a major cause of fungal meningitis, comprises two varietiesC. neoformans var. grubii and var. neoformansthat occasionally hybridise. We aimed to resolve hybrid lineage structure, parental origins, and recombination mechanisms driving genome evolution in this pathogen. We analysed 197 C. neoformans genomes, including 13 newly sequenced isolates, using phylogenomic, population genetic, and long-read assembly approaches. Analyses included mitochondrial inheritance, admixture, loss of heterozygosity (LOH), and structural variation to identify hybridisation signatures. We identified 73 hybrid isolates forming two previously unrecognised groups (H1 and H2) with distinct ecological origins and parental ancestry. H1, comprising exclusively clinical isolates, showed limited admixture but high nu cleotide diversity, consistent with ancient hybridisation and backcrossing. H2, primarily environmental or laboratory-derived, exhibited extensive admixture between VNI and VNIV lineages. Both groups displayed pervasive aneuploidy, large-scale LOH, and mosaic haplotype inheritance consistent with parasexual recombination. Phenotypic assays revealed substantial variability in growth and virulence, highlighting functional impacts of hybrid genome plasticity. Our study provides the first evidence of parasexual recombination in C. neoformans, revealing a composite lifecycle that integrates mitotic and meiotic processes. Parasex emerges as a key mechanism shaping genome evolution, population diversity, and pathogenic potential in this globally important human pathogen.

72 – The role of Eisosomal proteins in pathogenesis of Cryptococcus neoformans

Lukasz Kozubowski

Tejas Mahendra Patel

Clemson University

Cell wall and plasma membrane (PM) contribute to fungal pathogenicity. PM-associated eisosome complex plays key roles in PM organization, stress response, and cell wall synthesis. Eisosomes have been described in the ascomycetes phylum; however, no study has been conducted yet to understand eisosomes role in Cryptococcus neoformans. In Saccharomyces cerevisiae, Pil1 and Lsp1 proteins are the core components of eisosomes that shape the curvature of the PM based on their conserved AH-BAR domains. Our results revealed two localization patterns for fluorescently tagged Pil1 and Lsp1 homologues in C. neoformans: the canonical punctate localization to the PM and a novel localization to the mother-bud neck. The pil1 and lsp1 mutants exhibited inverse sensitivity to various stress conditions suggesting an unexpected mechanism of how Pil1 and Lsp1 cooperate in stress response. Cells lacking Lsp1 exhibited fewer and more intense punctate signals of Pil1-GFP as compared to tho se found in the wild-type, suggesting a role of Lsp1 in eisosome formation and/or dynamics. Interestingly, elimination of Lsp1 had similar consequences on the localization of the septin Cdc12-GFP, which forms filamentous punctate assemblies at the PM specifically when cells are exposed to 37ºC. As the well-described function of septins is at the mother-bud neck during cytokinesis, our results point to a novel connection between septins and eisosome components that involves PM homeostasis and cytokinesis. Strikingly, the double mutant (pil1lsp1) revealed defects in two major virulence factors, polysaccharide capsule and secretion of urease, pointing to a critical contribution of eisosomes to pathogenicity of C. neoformans.

73 – Ubiquitin mediated stress adaptation in Cryptococcus neoformans

Alejandro Antonia

A.L. Antonia, M. du Plooy, J.A. Alspaugh

Duke University Hospital

As an opportunistic fungal pathogen, Cryptococcus neoformans survives in widely varied physical environments. This ability to adapt, particularly to high temperature and stressors in human hosts, allows the yeast to survive, proliferate, and cause severe invasive infections. Underlying this stress adaptation, C. neoformans precisely regulates cell structure and metabolism in part by the post-translation modification of ubiquitination. A ubiquitin ligase enzyme, Rsp5, is essential for C. neoformans to grow under mammalian stressors ranging from temperature to pH. This broad range of functions is mediated by pleiotropic interactions between Rsp5 and diverse substrates. However, the mechanisms of Rsp5 regulation and specificity in response to unique stressors is incompletely understood. Here we use a co-immunoprecipitation based proteomics screen to identify novel C. neoformans E3-ligase regulatory proteins under host-stress conditions. The two most highly enriched Rsp5 inter acting proteins are previously uncharacterized in C. neoformans, but structural homologs in other model organisms regulate ubiquitin ligase activity and function. We demonstrated that C. neoformans mutant strains lacking these likely novel Rsp5 adaptor proteins have altered growth at 39ºC. Finally, because the homologues of these novel Rsp5 adaptors are involved in free radical damage response, we tested and found that C. neoformans Rsp5-dependent thermotolerance is also mediated partly by free radical stress. These studies provide novel insight into how C. neoformans uses ubiquitination to survive in the hostile host environment. Defining the molecular basis for invasive fungal pathogens to survive in the infection environment will help understand how they cause disease and discover potential therapeutic targets.

74 – A modified chemical-induced protein dimerisation assay identifies key phosphatase interactions for spindle checkpoint silencing in Cryptococcus neoformans

Steffin Rodrigues

Dr. Taylor Dyba, Dr. Ardra Suresh, Professor Kevin Hardwick

Institute of Cell Biology, School of Biological Sciences, University of Edinburgh

During metaphase, incorrect or misoriented attachment of spindle microtubules (MT) to the kinetochore (KT) activates the widely conserved spindle checkpoint, whose signalling prevents cell cycle progression into anaphase. The assembly of the signalling complex, the mitotic checkpoint complex (MCC) and the interactions between the subunits have been widely studied in humans and model ascomycetes. Little is known of the checkpoint dynamics in basidiomycetes, including in human fungal pathogen Cryptococcus neoformans. In this study we have adapted the SynCheck assay previously developed in Schizosaccharomyces pombe, which utilises abscisic acid (ABA) to chemically dimerise two proteins tagged with either a PYL or ABI signalling domain, bringing key checkpoint components together allowing for analysis of checkpoint activation and silencing. To adapt the SynCheck assay for C. neoformans, we tagged the core checkpoint kinase Mps1 with the ABI signalling domain, and either Spc105 or Bub1 with the PYL domain. In the presence of ABA, Mps1 phosphorylation of either Spc105 or Bub1 was sufficient to activate checkpoint arrest, whilst mutating the newly identified key phosphorylation residues in Spc105 impaired checkpoint arrest. Mutation of the phosphatase binding sites on Spc105 and Bub1 impaired checkpoint silencing: cells remained arrested longer than wildtype cells, demonstrating the need for both PP1 and PP2A phosphatase activity in C. neoformans checkpoint silencing. We have adapted the SynCheck assay for use in C. neoformans and through a proof of principle experiment, elucidated some of the key components of phosphorylation regulation, MCC signalling, and checkpoint silencing.

75 – Antifungal drugs induce cell wall remodelling in Cryptococcus neoformans

Eduardo Rafael Galue Mozo

Hilaire Irere 1, Lenka Koptasikova 2, Christian Hacker 2, Liliane Mukaremera 1

Medical Research Council Centre for Medical Mycology at The University of Exeter, University of Exeter, Exeter, United Kingdom.

Background and aim The fungal cell wall is a dynamic structure essential for cellular integrity and adaptation to stress including antifungals. It is not known whether Cryptococcus modifies it cell wall in response to antifungal drugs. This study aimed to test if C. neoformans changes its cell wall composition when exposed to antifungal drugs. Methods C. neoformans H99 was exposed to fluconazole, amphotericin B, 5-fluorocytosine (5FC), or different drug combinations for 24hours. Then, its cell wall structure was examined using electron microscopy. In addition, fluorescence microscopy was used to measure cell wall components including chitin, exposed chitooligomers, chitosan, and mannan. Results Electron microscopy showed an overall thickening of the C. neoformans cell wall following exposure to antifungal drugs. Quantitative fluorescence microscopy revealed that in the presence of fluconazole, exposed chitooligomers and mannan levels increased, while chitin and chitosan levels remained unchanged. Amphotericin B exposure resulted in reduced chitin levels, with minor changes in exposed chitooligomers, chitosan, and mannan. In contrast, 5FC caused a pronounced reduction in chitosan levels, while chitin, chitooligomer exposure, and mannan content showed only modest changes. Drug combinations produced additive effects: fluconazole combined with amphotericin B partially restored chitin levels while maintaining increased chitooligomer exposure and mannan abundance; fluconazole combined with 5FC increased mannan levels alongside sustained chitosan reduction. Conclusions C. neoformans adapts to antifungal presence by remodelling its cell wall structure and composition. This cell wall remodelling can contribute to the development of antifungal resistance and immune evasion of C. neoformans.

76 – Disruption of the cell wall integrity pathway drives the production of apoptotic-like vesicles in Cryptococcus neoformans

Cassandre Deculty

Patricia Flamant (1), Frédérique Moyrand (1), Pierre-Henri Commere (2), Sandrine Schmutz (2), Rayen Elj (3), Magdalena Gil Taran (3), Mariette Matondo (3), Gérard Pehau-Arnaudet (4), Adeline Mallet (4), Guilhem Janbon (1) and Isabelle Mouyna (1)

RNA and Extracellular Vesicles, Institut Pasteur, Université Paris Cité, Inserm U1359 Mycologie Moléculaire et Médicale, Paris, FRANCE

Extracellular vesicles (EVs) are lipid bilayer-bound particles released by cells into the extracellular environment and are involved in many biological processes, including intercellular communication, antifungal resistance and virulence. Unlike in mammals, the mechanism by which Cryptococcus neoformans EVs are secreted out from the cells remains unknown and our knowledge on EV production regulators is still limited. To identify regulators of EV biosynthesis, we performed a screening on a kinase mutant library for EV production in C. neoformans. We identified the Cell Wall Integrity (CWI) pathway kinases Bck1, Mkk2 and Mpk1 as negative regulators of EV production. Accordingly, we demonstrated that the three mutants, bck1, mkk2 and mpk1, produce a higher amount of EVs than the wild-type strain. Moreover, cryo-electron microscopy and nanoflow cytometry revealed that EVs produced by the CWI mutant strains are larger, and less decorated with mannoproteins than those produced by the wild-type strain. In agreement with these observations, proteomic analysis showed that the EV proteome of the mpk1 mutant is extensively remodeled, containing fewer GPI-anchored proteins and higher levels of ribosomal and histone proteins compared to wild-type EVs. Finally, annexin V labeling revealed increased phosphatidylserine (PS) exposure on EVs associated with CWI gene mutations. Overall, these results suggest that CWI mutant produce a subpopulation of EVs sharing characteristics with apoptotic vesicles previously described in mammals. They also indicate that C. neoformans, and fungi more generally, can produce distinct EV subtypes that may play different roles during the infection.

77 – Expanding insight into the Cryptococcus neoformans proteome and morphotypes through proteomic and covariation analyses

Louise Chapman

Kevin Hardwick, Georg Kustatscher

University of Edinburgh

As we develop molecular toolkits to study Cryptococcus neoformans, an area that is lacking is a detailed annotation of its proteome. With 60% of C. neoformans proteins still uncharacterised, this is an area sorely in need of further attention. High-throughput studies have aided this endeavour through co-expression analyses and phenotypic screens; however, to date, no high-throughput studies have incorporated proteomics. Here I have established a workflow for proteomics covariation analysis, a method which can be used to identify functional connections between proteins by measuring how their abundances fluctuate together over different conditions. We aim to complement the exciting findings from previous high throughput screens, by using this proteomics workflow. In the process, we hope to discover basidiomycete specific pathways which will aid in the identification of future drug targets. I am also developing methods to enrich for clinically relevant morphotypes, titans and titanides, which remain uncharacterised in their transcriptome and proteome. Through comparisons with their typical yeast morphotype, we hope to identify morphotype specific markers and pathways which would aid further study. This work will contribute towards a growing understanding of how a master of stress adaption, C. neoformans, promotes successful infections and improve our appreciation of a world of basidiomycete specific protein co-regulation networks that we are only just beginning to uncover.

78 – Titan Cell Production Increases In Vivo Diversity and can Enhance Antifungal Drug Resistance.

Kimberly Martinez

Andy Tawney, Kirsten Nielsen

Virginia Tech

Titan cells are large polyploid cells that play a role in virulence of C. neoformans and make up about 30% of the cell population in vivo. Previous in vitro studies showed that titan cells have an increased probability of randomly generating progeny with increased resistance to the antifungal drug fluconazole. Since titan cells are a minority of the in vivo cell population, we then asked is titan cell production biologically relevant for in vivo development of antifungal drug resistance? To address this question, we utilized the mouse inhalational model of cryptococcosis and infected mice with the laboratory reference strain KN99 that generated titan cells, and a congenic gpr4 gpr5 deletion strain with minimal titan cell production. Mice were either left untreated or treated with 10 mg/kg of fluconazole. At 14 days post infection, lungs were homogenized and serially plated in the presence of increasing concentrations of fluconazole. We then enumerated colony forming units (CFU) and colony size to quantify levels of fluconazole resistance (IC50), heteroresistance, and tolerance. No difference in IC50 or tolerance was observed with and without titan cell formation. However, higher rates of heteroresistance were observed with titan cell production that was independent of in vivo fluconazole exposure. These data suggest that in vivo titan cell formation is biologically relevant through the generation of increased population diversity that can influence antifungal resistance.

79 – DNA and histone methylation govern genome stability and virulence in Cryptococcus neoformans

Connor Larmore

Jun Huang, Vikas Yadav, Sheng Sun, and Joseph Heitman

Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC, USA

Cryptococcus neoformans is a basidiomycete yeast and WHO-designated critical-priority fungal pathogen responsible for ~194,000 annual cases of cryptococcal meningoencephalitis. While epigenetic regulation has been implicated in multiple biological processes in Cryptococcus, our understanding of these mechanisms remains limited. Here, we investigated the functions of two repressive epigenetic marks, DNA methylation (5mC) and histone H3 lysine 9 dimethylation (H3K9me2), which are enriched in repeat-rich regions of the genome, including the centromeres and telomeres, and are deposited by the DNA methyltransferase Dnmt5 (DMT5) and the histone methyltransferase Clr4 (CLR4), respectively. Employing mutants engineered in the H99 reference strain and in Bt65, an RNAi-deficient clinical isolate with a massive transposon burden, we found that disrupting 5mC or H3K9me2 resulted in defects in nuclear segregation and cell division, elevated rates of chromosome loss and aneuploidy, compromised thermotolerance, and attenuated virulence during murine infection. Disrupting 5mC or H3K9me2 also increased transposon expression, although no differences in transposon mobility were detected by the assays employed here. Furthermore, we identified strain-dependent negative genetic interactions between 5mC and H3K9me2 with RNA interference (RNAi), another genome defense mechanism. Together, these findings support a model in which 5mC and H3K9me2 play critical roles in maintaining genome stability and contribute to fitness and pathogenesis in C. neoformans.

80 – Fluconazole stress and MSH1 mutation promote resistance and phenotypic shifts in Cryptococcus neoformans

Matheus Janeck Araujo

Grace Paul, Sandra Catania, Rhys Farrer, Dora E. Corzo-Leon, Elizabeth R. Ballou.

MRC Centre for Medical Mycology, University of Exeter, Exeter, UK

Despite global treatment guidelines recommending combination therapy for cryptococcosis, fluconazole monotherapy remains common in resource-limited settings and is associated with increased risk of resistance. One mechanism for resistance observed in clinical isolates is the occurrence of hypermutator strains, exemplified by mutations in nuclear DNA Mismatch Repair genes MSH2 and MSH6. Recently, we identified a multi-drug resistant clinical isolate carrying mutations in the mitochondrial mismatch repair gene MSH1.Here, we investigated how stress adaptation and antifungal resistance are impacted by disruption of MSH1. Using CRISPR-Cas9 mediated genome editing, we generated a scarless msh1 and reconstituted either the wild-type and the clinical point-mutated MSH1 in a H99O background. Strains were subjected to experimental evolution in the presence of fluconazole (32ug/mL) for 1 month. We assessed phenotypic impacts before and after the experimental exposure including via g rowth assays, minimum inhibitory concentration testing, XTT metabolic activity assays, melanin production assays, titan cell induction, and cell wall staining. Whole-genome sequencing was performed to confirm the strains identity and genetic alterations. Notably, msh1 strains and clinical reconstituted mutants were initially sensitive to fluconazole, but displayed spontaneous tolerance to high doses of flucytosine and increased resistance to Amphotericin B within 1 month. This suggests a two-hit model of cross-resistance, driven by genomic instability, despite intact nuclear DNA Mismatch Repair. These findings highlight the interplay between mitochondrial repair pathways, stress adaptation, and the emergence of antifungal resistance induced by fluconazole monotherapy in C. neoformans.

81 – Deciphering the Molecular Architecture of Outer Kinetochore Assembly in Cryptococcus neoformans

Shiyon Anto Vadakkan

Vimal Das, Bethan Medina-Pritchard, Maria Alba Abad and A. Arockia Jeyaprakash

University of Edinburgh

The kinetochore composition of the opportunistic human fungal pathogen, Cryptococcus neoformans, is significantly divergent from that of canonical budding yeast and human systems due to the lack of the whole Constitutive Centromere Associated Network (CCAN), except for CENP-C, which is replaced by a novel kinetochore protein called Bridgin. Despite its divergence, the outer kinetochore components such as, Mis12 complex (Mis12c), Dam1 complex (Dam1c) and Ndc80 complex (Ndc80c), appeared to be conserved across fungal species. In well studied systems like Saccharomyces cerevisiae, Dam1c assembles in a shoulder-to-shoulder conformation, by staple motifs in the Dam1 subunit that promotes oligomerization, forming a ring-like structure around the microtubules. The Ndc80c bridges microtubules to the Mis12c by extending and folding across the outer surface of the Dam1c. This arrangement allows the Dam1c and Ndc80c to work together to establish strong and stable kinetochore-mic rotubule attachments. To ensure proper bi-orientation is achieved, this interaction is regulated by Aurora B kinase (Ipl1 in C. neoformans), which can phosphorylate key residues at the Dam1cNdc80c interface, destabilizing erroneous kinetochore-microtubule attachments and promoting their rapid detachment and reattachment.

Here, we aim to characterize the organization of Dam1c, Ndc80c and their interactions with microtubules in C. neoformans, using integrated structural approach combining Cryo-EM and X-ray crystallography. Elucidating the molecular architecture and the phospho-regulation of the outer kinetochore components will shed light on how these seemingly conserved complex have adapted to a lack of CCAN, and is essential to uncover potential therapeutic vulnerabilities of this fungal pathogen.

82 – Comparison of Bruker Biotyper Sirius, VITEK MS PRIME, and ASTA MicroIDSys MALDI-TOF MS Systems for Cryptococcus Species Identification Across Extraction Protocols

Eun Jeong Won

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA; Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background and aims: Accurate and rapid identification of Cryptococcus spp. is critical for patient care. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) enables yeast identification; however, its performance varies with instrument and database coverage. We compared the performance of three MALDI-TOF MS systems Bruker Biotyper Sirius (Sirius; Bruker Daltonics), bioMérieux VITEK MS PRIME (PRIME; bioMérieux), and ASTA MicroIDSys (ASTA; ASTA Inc.)in detecting Cryptococcus species across extraction protocols. Methods: In total, 51 C. neoformans, two C. deuterogattii, five Vanrija humicola, and one Naganishia liquefaciens clinical isolates were analyzed. Each isolate was first tested after on-plate formic acid extraction; isolates failing identification were retested after repeat on-plate and in-tube extractions. Results were categorized as correct, incomplete, incorrect, or no identification against ribosomal DNA sequencing results. Results: After initial on-plate extraction, correct identification rates were 86.4% for Sirius, 88.1% for PRIME, and 69.5% for ASTA. After repeat on-plate extraction, the accuracy of ASTA increased to 81.4% (P = 0.016). Additional in-tube extraction did not significantly improve identification results across platforms, although PRIME outperformed ASTA (P = 0.012). All platforms reliably classified C. neoformans (correct identification rates: 94%96%) after additional on-plate extraction; discrepancies occurred mainly in uncommon species, possibly due to gaps in database coverage. Conclusions: Sirius and PRIME demonstrated robust performance for C. neoformans identification and C. gattii complex differentiation, outperforming ASTA for relatively rare species, owing to differences in database coverage. Single on-plate extraction is sufficient in clinical practice; however, continued database expansion is essential to maximize MALDI-TOF MS performance.

83 – Putting microglia to bed: Cryptococcus neoformans induces a senescence-associated transcriptional profile in the HMC3 microglia cell line.

William Newton

Dr Sebastian Müller, Dr Jonathan Witton, Dr Elizabeth Ballou, Dr Carolina Coelho

University of Exeter

Background and aims:

Cryptococcus neoformans is a neurotropic fungus known to subvert immune challenge by microglia, the brain resident macrophage, and utilize them as reservoir for infection (Mohamed et al., 2023). Single-cell transcriptomic analysis of brain tissue from murine models of C. neoformans and Candida albicans cerebral infection demonstrated significantly delayed activation of microglia to C. neoformans by up to 14 days, in comparison to 4 days with C. albicans (Reyes et al., 2025). However, the mechanisms by which C. neoformans subverts microglial antifungal responses remains poorly understood. Here, we used a transcriptomic approach to dissect microglial responses in vitro to C. neoformans and C. albicans and identify species-specific pathways.

Methods:

The human microglia HMC3 cell line was infected in duplicate at an MOI 2:1 (yeast:microglia) with serum-opsonised H99E C. neoformans morphotypes (rich-media, titan-induced, capsule-induced, seed cells) or SC5314-efg1Δ/Δ/cph1Δ/Δ C. albicans ± 100 U/mL interferon-γ (IFNγ) for 6- or 24-hours in biological triplicate. RNA was isolated by triZOL and a Direct-ZOL miniprep kit. Library preparation and Illumina PE150 sequencing was performed by Exeter Sequencing Service. Adaptors were removed from reads by Trimmomatic and aligned using Salmon before differential expression analysis by DESeq2 with help from ALVA genomics.

Results:

We identified 192 genes differentially expressed by microglia in response to C. neoformans, of which 79% also differentially regulated in response to C. albicans. Feature-wise clustering identified genes inversely expressed in response to C. neoformans and C. albicans with 546 DEGs exhibiting a fold-change > 1.5. Pathway enrichment analysis showed C. neoformans infection uniquely upregulated Hippo, Notch, and Wnt signalling pathways and negatively regulated cellular transcription by RNA polymerase II. Screening 81 cell-cycle marker genes against our dataset identified an upregulation of G0-associated genes with C. neoformans infection, whereas these were downregulated by C. albicans. Further analysis of quiescence- and senescence-associated genes showed differential expression of 34 genes from both fates but 64% of upregulated genes being senescence-associated. Microglial expression of NADH dehydrogenase was unaffected by C. neoformans, and a resazurin assay confirmed maintenance of microglial metabolic viability, both indicative of senescence.

Conclusions:

These data are consistent with a model in which C. neoformans induces a senescence-associated transcriptional profile in HMC3 microglia at 24 hours. This is supported by a maintenance of microglial metabolic viability during infection. Future work should perform further phenotypic assays to assess microglial proliferation, β-galactosidase activity, and secretion of senescence-associated cytokines.

84 – IL-5Rα deletion controls cryptococcal meningoencephalitis development

Lander De Herdt

Agustin Reséndiz Sharpe1, Eliane Vanhoffelen1, Tine Van Win2, Fabrice Ajonglefac Nkengeh7, Kiréna Mollers3, Katrien Lagrou2,9, Philippe van den Steen4, Christophe J. Desmet3, Max Nobis5,6, Jan Remsik7, Colinda Scheele8, Greetje Vande Velde1

Biomedical MRI, Department of Imaging and Pathology, KU Leuven, 3000, Leuven, Belgium

Main: Cryptococcal meningoencephalitis (CM) is the result of CNS entrance of Cryptococcus neoformans (CN). Current treatment options are limited and result in long-lasting side-effects post-infection. Better understanding of the host immune response is necessary to develop immunomodulatory treatments. Results: To uncover immunological drivers of CN extrapulmonary dissemination and CNS entrance, we used an intranasal mouse infection model, advanced in vivo microscopic and mesoscopic imaging, proteomics, and transcriptomics to build a 3D spatiotemporal map. We found that pulmonary infection induced eosinophil expansion in the early stage of disease. Degranulating eosinophils were recruited in airways and alveoli, affecting the integrity and morphology of the airway epithelium. Deletion of the eosinophil lineage protein IL-5Rα resulted in lower CNS fungal burden. Mice lacking IL-5Rα had significantly lower expression of proteins associated with eosinophil degranulation, activation, antimicrobial killing, and airway epithelium distress, indicating a detrimental role of eosinophil activation during pulmonary cryptococcosis. Interestingly, IL-5Rα deficiency resulted in an increased IFN-ɣ response during lung infection prior to brain infection, but resulted in lower IFN ɣ levels once brain infection was established. This suggests an ambiguous role of IL-5Rα regulated IFN-ɣ in a time dependent manner during CM development. Future perspectives: We uncover IL-5Rα as novel potential therapeutic target for cryptococcosis. To further develop IL-5Rα targeting as immunomodulatory treatment strategy, we are unravelling the underlying molecular mechanism of IL-5Rα expressing cells through scRNA-seq of bronchoalveolar lavage fluid, blood, and leptomeninges, with data analysis and validation of results with functional in vivo and in vitro experiments currently underway.

85 – TNF-α, but not IFN-ɣ, mediates CD4+ T cell migration to the brain in a cell-intrinsic manner during Cryptococcus neoformans infection

Meiqing Shi

Department of Veterinary Medicine, University of Maryland, College Park, Maryland, USA

CD4+ T cell depletion in HIV/AIDS patients is believed to cause brain infections of Cryptococcus neoformans. Paradoxically, the rapid reconstitution and migration of CD4+ T cells to the brain in HIV/AIDS patients co-infected with C. neoformans after the initiation of antiretroviral therapy is associated with immune reconstitution inflammatory syndrome. Thus, recruitment of CD4+ T cells to the brain is crucial for both protection and disease progression during C. neoformans infection, but the underlying mechanisms remain incompletely understood. We studied the mechanism of CD4+ T cell migration to the brain using flow cytometry. The results show that clearance of C. neoformans in the brain of mice correlates with the accumulation of CD4+ T cells and the secretion of TNF-α and IFN-ɣ. Disruption of TNF-α receptor signaling enhances IFN-ɣ production in the brain, whereas the absence of IFN-ɣ receptor signaling results in a higher secretion of TNF-α. Disruption of TNF-α receptor signaling impairs CD4+ T cell recruitment to the infected brain. By contrast, disruption of IFN-ɣ signaling leads to enhanced CD4+ T cell accumulation, which can be prevented by TNF-α neutralization. Adoptive transfer experiments suggest that TNF-α acts directly on CD4+ T cells to promote their recruitment to the brain. Collectively, our data demonstrate that TNF-α but not IFN- ɣ, drives CD4+ T cell migration to the brain in a cell-intrinsic manner during C. neoformans infection.

86 – The Mannose Receptor (MR) on CD169+ myeloid cells modulates the generation or maintenance of anti-fungal Tbet+ stem-like progenitors

Chinaemerem Uju Onyishi

Eric V. Dang

Ragon Institute of Mass General, MIT, and Harvard, Cambridge, MA, 02319, USA

The mannose receptor (MR) is a C-type lectin receptor broadly expressed on myeloid cells and often considered a marker of immunosuppressive M2-like macrophages. However, recent data have identified immunostimulatory roles for MR, highlighting its complexity and context-dependent functions. Hence, we sought to investigate the precise functional role(s) of MR in modulating T cell fate decisions using the highly mannosylated, CD4 T cell-dependent fungal pathogen Cryptococcus neoformans as a model. At 10 days post intranasal infection with KN99alpha C. neoformans, MR-deficient mice exhibited decreased Tbet+ Th1 cells in the TCF1high stem-like progenitor compartment in both the lungs and lung-draining lymph nodes (ldLN), while terminal effector Th1 cells and other Th subsets were unaffected. Using mixed bone marrow chimeras to selectively deplete MR from specific myeloid populations, we found this defect occurred only when MR was deleted from CD169+ cells, but not from cDC1s or cDC2s. Furthermore, loss of MHCII within the CD169+ compartment similarly impaired Tbet+ progenitor generation. Notably, MR+ CD169+ cells in the ldLN expressed IL12p40 and formed direct contact with CD4 T cells, identifying these cells as a previously unrecognized bona fide antigen-presenting cell population that utilizes MR to selectively license Tbet expression on stem-like progenitor cells. Finally, to determine functional relevance, we infected mice with the gcs1delta C. neoformans strain which establishes a chronic infection in which protection is mediated by Th1 immunity. We found that MR-deficient mice had decreased survival compared to controls, suggesting that MR-dependent Th1 progenitor maintenance is required for effective long-term antifungal immunity.

87 – Central role of regulatory T cells in protecting brain from inflammatory damage in cryptococcal meningoencephalitis

Michal Olszewski

Hailong Li1,2,3, Rylan Hissong1,2, Kristie Goughenour1,2, Jessica C. Hargarten4, Brian Song2, Heineken Q. Daguplo2, Anutosh Ganguly2, Peter R. Williamson4, Jintao Xu1,2

University of Michigan Medical School and Ann Arbor VA Health System, Department of Veterans Affairs Health System, Ann Arbor, Michigan, USA

Unchecked T-cell inflammation in the brain contributes to the progression of cryptococcal meningoencephalitis (CM), including neuronal damage and apoptosis. We examined the function of regulatory T-cells (Tregs) during CM in maintaining immune balance and their precise role/mechanisms in preventing pathological neuroinflammation. We found that Tregs are present in the CSF of CM patients and accumulate in the mouse brain over the course of disease progression, albeit with some delay compared to the effector T-cells. Treg depletion resulted in exacerbated neurological symptoms and increased mouse mortality to a 100%, despite a reduction in fungal burden. This was due to increased Th1-inflammation, neuronal damage and apoptosis. We found that Treg recruitment into the brain largely depended on CCR8-CCL1. Tregs were the major source of anti-inflammatory IL- 10, of which deletion phenocopied Treg depletion in our CM model. Tregs, in CM brain, were also an important source of Amp hiregulin (Areg), a trophic, neuroprotective factor involved in tissue repair. Interestingly, Treg-restricted knockdown of Areg suppressed IFN-ɣ production by Teffs, but ultimately did not change the symptoms, pathology, or mouse survival. Finally, Treg-expanding immunotherapy with low-dose IL-2 vastly improved the CM-mouse neurological score, preserved survival, and reduced inflammation, neuronal damage, and apoptosis. In conclusion, CCR8-CCL1-recruited Tregs are critical for maintaining immune balance in the brain during CM. Harnessing these cells could be exploited therapeutically as a strategy to mitigate CM immunopathology and fine-tune the protective immune responses in the brain for optimal clinical outcomes.

88 – Role of H2-K1 and Metabolism in Defining Innate Immunity to Cryptococcus neoformans

Ayesha Nair

Karen L. Wozniak

Oklahoma State University

Cryptococcus neoformans, the etiological agent of cryptococcosis, can survive and replicate within host immune cells, contributing to the development of cryptococcal meningitis. Within the lung, C. neoformans interacts with innate immune cells, including macrophages and dendritic cells (DCs). Our previous work showed that Ly6C- monocyte-like macrophages restrict fungal proliferation, whereas CD11b+ DCs promote fungal growth. Transcriptomic analysis revealed differential expression of the MHC class I gene H2-K1, Calreticulin (Calr), and multiple metabolic genes, leading us to hypothesize that antigen presentation and metabolic pathways regulate the intracellular fate of C. neoformans. To test this hypothesis, we performed siRNA-mediated knockdown (KD) of H2-K1 in J774 macrophages and bone marrowderived DCs (BMDCs). H2-K1 KD significantly reduced antifungal activity in both J774 cells and BMDCs. Additionally, H2-K1 KD resulted in reduced Calr expression suggesting H2-k1 gene has downstream effects in the pathway. We also observed a significant decrease in reactive oxygen species (ROS) production in H2-K1 KD cells. Notably, H2-K1 knockdown did not significantly alter glycolytic pathway, suggesting that H2-K1mediated ROS production and metabolic reprogramming function through independent mechanisms. Further investigation of the pentose phosphate pathway (PPP) revealed that PPP inhibition significantly reduced cryptococcal CFUs while increasing ROS levels. Ongoing studies are examining whether the reduced ROS production observed in H2-K1 silenced cells is PPP dependent. These findings indicate that the H2-K1 gene and metabolic reprogramming are critical for effe ctive antifungal responses. Our data provide a foundation for future studies aimed at targeting host immune pathways to enhance antifungal immunity.

89 – Investigating the meningeal immune response during Cryptococcus infection

Miranda Lumbreras

Estefany Reyes, Malia Dickhens, Mari Shinohara

Duke University

Cryptococcal meningoencephalitis (CM) is a lethal fungal infection characterized by inflammation of the meninges and brain parenchyma. While pulmonary immune responses to Cryptococcus are well studied, immune mechanisms within the central nervous system (CNS) remain poorly understood. Using a CM model with C. deneoformans 52D strain, we previously observed that although fungal dissemination to the brain occurs as early as one day following intravenous infection, CNS-resident immune cells require approximately two weeks to mount a detectable response. Notably, microglial activation does not occur through direct fungal sensing but instead depends on T cell infiltration into the brain, suggesting that microglia may not function as primary immune sentinels in this context.

We therefore hypothesized that other cell types initiate CNS immune responses. We focused on border-associated macrophages (BAMs), a population of resident myeloid cells in brain border tissues. We found that BAMs become activated as early as day 2 post-infection. This activation is followed by recruitment of monocytes and subsequently T cells, which produce IFN-ɣ. Importantly, this sequence and the duration of immune events appeared to be specific to Cryptococcus, as it was not observed in a Candida CNS infection model. These findings demonstrate that BAMs play a critical role in initiating leukocyte infiltration into the brain, ultimately leading to microglial activation.

90 – The genetic and environmental regulation of fungal zincophore systems across the Dikarya

Charlie Holt

Dr Liping Xiong (2), Dr Elena Roselletti (1), Prof. Joseph Heitman (2), Prof. Elaine Bignell (1), Dr Duncan Wilson (1)

MRC CMM, University of Exeter

Zinc is a nutritionally critical trace metal, essential to all living organisms. During fungal infection, mammalian hosts employ nutritional immunity (restricting trace metal availability) to limit infection progression and severity. To overcome this, pathogenic fungi employ multiple virulence strategies, including mechanisms for trace metal acquisition. The fungal scavenging of zinc in some fungi, including some Cryptococcal species and Candida albicans, is facilitated by utilising the Pra1-Zrt101 zincophore mechanism to overcome zinc-limited environmental niches.

This work investigated the functional, regulatory and evolutionary relationships of the Pra1-zincophore among Ascomycete and Basidiomycete fungi. To build on current knowledge of the Pra1-zincophore we studied C. albicans, Cryptococcus neoformans and Cryptococcus amylolentus using a combination of in silico modelling, in vitro assays and in vivo infections. To precisely define the regulatory control of PRA1 expression in response to pH and zinc, in vitro assays demonstrate PRA1 expression in neutral-alkaline pH but suggest a pH-zinc fine-tuning of expression between species. In comparison to C. albicans, C. amylolentus displays a four-fold more sensitive responsiveness to supplemented zinc in repressing PRA1. We also investigated the role of Pra1 to virulence and neutrophils.

We conclude, the Pra1 zincophore is functionally conserved across the Dikarya, highlighting the potential therapeutic role of targeting Pra1 during infection. Additionally, we conclude the status of the fungal zincophore loci impacts fungal immune evasion properties.

91 – Time-Dependent Antibody Signatures of Cryptococcal Disease After Kidney Transplant

Hyunah Yoon

Johanna Rivera, Enver Akalin, Liise-anne Pirofski

Albert Einstein College of Medicine

Background and aims: Cryptococcal disease (CD) remains a high-mortality opportunistic infection after kidney transplant (KT), typically presenting during maintenance immunosuppression (IS) when immune reconstitution is heterogeneous. No validated immune markers risk-stratify KT recipients for CD. We evaluated whether total/antigen-specific antibody profiles differ by CD status and time post-KT as a surrogate for cumulative IS exposure. Methods: Plasma from three KT cohorts (n=58) was analysed: no-CD (n=29), pre-CD (n=10; prior to CD diagnosis), and CD (n=19; after diagnosis); stratified early (<1 year) or late (>1 year) post-KT. Total IgM, IgA, IgG1, IgG2, and cytokines quantified by Luminex; antigen-specific IgM/IgG to capsular glucuronoxylomannan (GXM) and laminarin (β-glucan proxy) by ELISA. Results: Median age was 58 years; 75% male. CD occurred 2.2 years post-KT; sampling ~33 days later. Compared with no-CD, CD had hypogammaglobulinemia, ~5-fold lower IgM (median 928?g/ml vs 4,441), ~4-fold lower IgG2 (482 vs 1,940); late CD remained depressed (IgM 775 vs 3,588; IgG2 651 vs 1,711). Within CD, antigen-responses declined from early to late (GXM-IgM 21.614.4; laminarin-IgM 16151; laminarin-IgG 304163), contrasting with stable/increasing levels in no-CD/pre-CD. Despite low total immunoglobulins, early CD had comparable/higher GXM-IgM and laminarin-IgM than early no-CD (21.6 vs 16.3; 161 vs 49.8) while GXM-IgG was lower (45.9 vs 121). IgM, laminarin/GXM-IgM correlated with IFNɣ, GM-CSF, and IL-17A in CD. Conclusions: CD after KT is associated with decreased IgM/IgG2 and time-dependent antigen-specific antibody trajectories during the expected period of CD4 reconstitution, suggesting distinct immune states driving disease, supporting marker-guided IS management. Small strata, heterogeneous sampling limit inference.

92 – Elucidating Dissemination of the Fungal Pathogen Cryptococcus neoformans Through Genetic Barcoding

Zanetta Chang

Tamara L. Doering

Division of Infectious Diseases, Washington University in St. Louis

Cryptococcus neoformans causes 194,000 cases of meningoencephalitis annually with very high mortality (~80%). Invasive cryptococcosis begins with inhalation of infectious organisms followed by dissemination throughout the host, especially to the central nervous system. The mechanisms underlying dissemination are essential for progression of disease yet remain poorly described. In a murine model of inhaled cryptococcosis I demonstrated that C. neoformans disseminates reliably to organs including the lungs, brain, liver, spleen, kidney, and blood. I then infected mice with a pool of five C. neoformans strains with varying drug resistance markers inserted at a genomic safe haven locus. The proportions of each strain remained stable in the lungs but became highly variable after dissemination to the brains of these animals, implying a bottleneck in fungal transit. To further interrogate the kinetics of dissemination I created a library of 125 barcoded, otherwise isogenic s trains in a wild-type background. This larger library eliminated the heterogeneity introduced by drug resistance markers and improved the resolution of our study. The library was pooled for intranasal infection followed by Sanger sequencing to determine barcode abundance in each organ at multiple timepoints during infection. A significant bottleneck was again revealed between the lung and brain in late-stage infection, and was found to vary by individual animal. In ongoing work I am performing an intravenous infection with the library to compare bottlenecks created by this alternative infectious route. Delineating cryptococcal dissemination will enable understanding of the clinical course and may create new avenues for therapeutic approaches.

93 – Assessment of cerebral cryptococcosis caused by different molecular sub-types in rodent models using correlations between in vivo imaging and histology

Dries Maes

L. Musetta (1); C. Preuße (2); Lara Roosen (1); W. Meyer (3); S. Helsper (1); K. Lagrou (4); W. Stenzel (2); U. Himmelreich (1)

KU Leuven

Molecular subtypes within the Cryptococcus species complexes manifest differently within the CNS, although the exact correlations between clinical and cellular manifestation are poorly understood. It was our aim to further characterize them in vivo5,6 as to deepen our understanding of the neuropathogenic aspects of cryptococcosis. Female BALB/c mice were intravenously infected (50.000 cells/100 L; n = 4) with an isolate representing one of the eight major molecular subtypes of C. neoformans (CN) (VN I-IV) and C. gattii (CG) (VG I-IV) as described by Meyer et al (Electrophoresis. 1999 Jun;20(8):17909). Mice were scanned weekly until the humane endpoints using a 9.4 T MRI scanner (Biospec 94/20, Bruker). Structural, diffusion weighted (ADC) and T2 relaxometry images were acquired and semi-quantified for assessment of lesion and meningitis formation, based on Vanherp et al (2020). Post-mortem histopathological analysis included a wide range of immunohistochemical markers to assess host-pathogen interactions. The CN/CG complex consists out of differently behaving subtypes deviating in manifestation/morbidity. We identified the capsular size to be the main virulence factor in the dissemination towards the CNS, as concluded from ADC and T2 relaxometry. Post-mortem histopathology confirmed these results, along with indications of CN/CG provoking an autoimmune-like host-reaction, linked to an immunosuppressed CNS phenotype. Lastly, we identified the choroid plexus as a main entrance point for cerebral dissemination. By implementing longitudinal, multiparametric MR acquisitions we gained new insights in the behavior and formation of cryptococcoma. Combined with histopathology, the in vivo imaging-based characterization of cryptococcosis allows for future investigation for an improved prognosis/treatment.

94 – Hir1, a subunit of the HIR complex, is required for iron regulation, cell wall integrity and virulence in Cryptococcus neoformans

Guanggan Hu

Christopher Lee, Jie Jiao, Xianya Qu, Kabir Bhalla, Eddy Sánchez-León, James Kronstad

Michael Smith Laboratories, The University of British Columbia, Canada

Iron is important for proliferation of C. neoformans, and acts both as a nutrient and as a signal to regulate the expression of the main virulence factors. We recently discovered a previously unidentified role of a transcription corepressor, Hir1, in iron acquisition and regulation, and in the virulence in C. neoformans. A hir1 mutant was originally identified by screening an Agrobacterium tumefaciens - mediated insertion library. In Saccharomyces cerevisiae and other fungi, Hir1 is a subunit of the HIR complex that is involved in regulation of histone gene transcription. Deletion of HIR1 in C. neoformans results in reduced capsule formation in low iron capsule-inducing media, and increased sensitivity to elevated temperatures. Furthermore, the hir1 deletion mutants exhibited reduced growth in iron-limiting media, poor growth on heme as the sole iron source, and increased sensitivity to the iron - chelating agent curcumin. Hir1 is localized in the nuclei of cells that grow in low or high iron conditions. Interestingly, preliminary results from a pull-down assay revealed that Hir1 is one of the possible interacting partners of the iron master regulator, Cir1, indicating that Hir1 may regulate iron acquisition by interaction with Cir1. Deletion of HIR1 also causes increased sensitivity to the azole drug, fluconazole, and compromised cell wall integrity. Lastly, a pilot virulence assay in a mouse inhalation model demonstrated that Hir1 is required for full virulence. In summary, our preliminary investigation identified a new role for Hir1 in iron acquisition, cell wall integrity and virulence in C. neoformans.

95 – Characterization of extracellular vesicles diversity in Cryptococcus neoformans: A new avenue for identifying disease markers and understanding host interaction.

Tonia DARGHAM

C-author affiliations:

Pasteur Institute Paris

Background and aims Extracellular vesicles (EVs) are fundamental mediators of intercellular communication and pathogenic processes across diverse organisms, including fungi such as Cryptococcus species, an opportunistic human pathogen. Although current structural and biogenetic models of fungal EVs have advanced the field, they often overlook the intrinsic heterogeneity of EV populations, which may underpin distinct biological functions. While EV subpopulations have been extensively characterized in mammalian systems, their diversity and immunomodulatory potential in fungi remain poorly understood. This study aimed to characterize EV heterogeneity in Cryptococcus neoformans and explore the functional relevance of distinct EV subpopulations. Methods Cryptococcus-derived EVs were isolated using an integrative strategy combining size-, density-, and weight-based separation techniques, enabling the resolution of discrete EV subpopulations. Proteomic profiling was performed to compare protein cargo across EV fractions and identify subpopulation-specific signatures. Results We identified multiple EV subpopulations with distinct biophysical properties and protein compositions. Notably, a previously uncharacterized small EV population was discovered, exhibiting a unique proteomic profile. Comparative proteomic analyses revealed that each EV subpopulation harbors distinct protein cargos, including candidates associated with immune modulation and fungal virulence. Conclusions This work reveals previously unrecognized layers of EV diversity in Cryptococcus neoformans. Ongoing functional studies using in vitro hostpathogen interaction assays are investigating how individual EV subpopulations differentially influence host immune responses, including cytokine production and immune cell activation. Together, these findings lay the groundwork for elucidating EV-mediated mechanisms of host interaction and support the potential development of EV-based biomarkers and therapeutic strategies targeting fungal pathogenic processes.

96 – Determination of hypothetical proteins role in Cryptococcal virulence

Marie-Gabrielle Ayika

Jennifer Geddes-McAlister

University of Guelph

Cryptococcus neoformans is an opportunistic fungal pathogen causing severe disease in immune compromised hosts. The increasing rates of disease prevalence and rising threat of antifungal resistance highlights the need to identify determinants of fungal infection and factors modulating host-pathogen interactions. Macrophages as the first line of defense against C. neoformans were used to uncover proteins important for their critical interaction. Herein, mass spectrometry-based proteomic profiling of co-cultured C. neoformans and bone marrow-derived macrophages identified multiple infection-associated candidates, including the uncharacterized protein, CNAG_03492. Based on reduced capsule size and thermotolerance, CNAG_03492 is hypothesized to influence cryptococcal virulence. To define this role, CNAG_03492 is being evaluated for changes in macrophage uptake and intracellular persistence using bone marrow-derived macrophages. In addition, in vivo studies using CNAG_03492Δ and complemented strains in BALB/c mice will be used to assess how CNAG_03492 affects fungal dissemination, organ-specific burden, and host survival. These findings will be complemented with in silico prediction modeling of target structure and interactions, as well as functional assays exploring global proteome disruption upon deletion of CNAG_03492. Together, these findings will explore the positioning of CNAG_03492 as a target for therapeutic development against cryptococcal disease.

97 – Determining the kinetics and consequences of germination in Cryptococcus spore-mediated disease

Nicolas Pereira

Megan McKeon, Christina Hull

University of Wisconsin-Madison, Department of Biomolecular Chemistry

Spores and yeast can both cause fatal cryptococcosis in mouse models of disease; however, spores from some backgrounds cause disease when their yeast parents cannot. Spores also differ from yeast in that they are readily engulfed by phagocytes and then disseminate from the lung via an apparent Trojan Horse mechanism. How differences in initial spore-phagocyte interactions influence disease outcomes are poorly understood. We hypothesize that lung escape is directly influenced by rate of germination and resulting changes in frequency of phagocytosis. To determine the timeline of germination in vivo, we infected mice intranasally with spores and evaluated germination progression over 24 hours. We discovered that germination in the mouse lung progressed at a rate similar to germination in vitro. However, in contrast to in vitro germination in which >99% of spores germinate, a sub-population of spores (~25%) failed to germinate in the mouse. To help determine the effects of host conditions on germination, we developed a lung homogenate (LH) medium and determined that it mimicked the in vivo germination phenotypes. To identify mechanisms of germination initiation we carried out RNA-seq of spores germinating in LH, which revealed specific upregulation of transcripts associated with stress tolerance and virulence. These data suggest that host-like conditions elicit a rapid and protective transcriptional response that facilitates survival and potentially pathogenicity. These findings lay the foundation for future experiments to determine how interactions with lung phagocytes change over time and how germination responses impact dissemination and disease outcomes.

98 – Dipeptidyl peptidase IV contributes to the pathogenesis of Cryptococcus neoformans

Flavia Coelho Garcia dos Reis

Haroldo C. Oliveira2, Daniel Zamith-Miranda3,4, Debora L. Oliveira1 , Brbara T. Bezerra1,5, Nicolly A. Sensato1, Cssia M. Souza1 , Juliana Rizzo6 , Lucas de O. Las Casas1,5 , Flora Bohner3,4, Bianca A. G. Sena1, Amanda C. Camillo-Andrade1,7, Marlon D. M. Santos1,7, Paulo C. Carvalho1 , Guilhem Janbon8, Joshua D. Nosanchuk3,4, Marcio L. Rodrigues1,9,10

Instituto Carlos Chagas - Fiocruz

We previously identified isoleucine proline isoleucine (IPI) as a small molecule associated with extracellular vesicles (EVs) in Cryptococcus. The administration of IPI controlled experimental cryptococcal infection through mechanisms that remain unknown. IPI is an inhibitor of dipeptidyl peptidase 4 (Dpp4), an enzyme that regulates insulin and glucose homeostasis in mammals and may also influence pathogenic processes in fungi. Based on these observations, we investigated the biological and pathogenic roles of cryptococcal Dpp4. We demonstrate that Cryptococcus neoformans synthesizes an active, surface- associated Dpp4 that is inhibited by IPI. Deletion of the Dpp4-encoding gene (CNAG_06416) abolished Dpp4 enzymatic activity and resulted in marked physiological alterations, including changes in the proteomic composition of whole cells and EVs, as well as increased accumulation of undivided cells at 37C both in vitro and in vivo. The dpp4Δ mutant was less lethal than wild-type (WT) cells in a Galleria mellonella infection model. In contrast, increased Dpp4 activity did not enhance lethality, as demonstrated by the ability of WT and 19 highDpp4-expressing mutant strains to kill G. mellonella. In a murine model, loss of Dpp4 significantly attenuated fungal virulence, delaying host mortality and reducing fungal burden, particularly in the brain. This finding is consistent with the observation that dpp4Δ cells were markedly less emicient at crossing endothelial and extracellular matrix layers than WT cells. Collectively, these findings establish DPP4 as an important regulator of cryptococcal physiology and pathogenesis, and support the concept that targeting Dpp4 may omer translational opportunities distinct from conventional antifungal strategies.

99 – Paradoxical microglial inactivation during cryptococcal infection of the brain

Amalia Awala

Maahir Kauchali 1, 2,3, Emily Ruth Higgitt 1,2,3, Anja de Lange 1,2,3, Alison Gifford 5, Joseph V Raimondo 2,4, Rachael Dangarembizi 1,2,3.

1 Division of Physiological Sciences, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa 2 Neuroscience Institute, Faculty of Health Sciences, University of Cape Town, Groote Schuur Hospital, Cape Town, South Africa 3 CMM AFRICA Medical Mycology Research Unit, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

Cryptococcal meningitis (CM) is a fatal fungal infection of the brain and remains a leading cause of HIV-related mortality worldwide. Brain injury in CM has traditionally been attributed to excessive inflammation; however, the cellular mechanisms underlying cryptococcal neuropathogenesis remain poorly defined. Particularly unknown is how microglia, the resident immune cells of the brain, respond to brain invasion by Cryptococcus neoformans (C. neoformans), the causative agent for CM. Here, we investigated microglial-fungal interactions using complementary rodent and human brain-based experimental systems. Human and rodent organotypic brain slices (OBSs) were infected with a GFP/mCherry-expressing H99 strain of C. neoformans, and host - pathogen interactions were examined using immunofluorescent labelling of microglia (IBA1). Microglial activation was measured using nuclear localisation of the transcription factor, nuclear factor for interleukin-6 (NF-IL6): a robust marker for inflammatory signalling. Immune responses in C. neoformans-treated slices were compared to untreated controls and to slices treated with the canonical immunogen- lipopolysaccharide (LPS). Inflammatory responses were further validated using single-nucleus RNA sequencing and multiplex cytokine profiling. Microglia readily internalised C. neoformans; however, fungal uptake was not accompanied by classical microglial activation. C. neoformans -treated slices showed minimal NF-IL6 activation, negligible pro-inflammatory cytokine release, and no induction of key inflammatory gene programs, comparable to untreated controls and markedly attenuated relative to LPS-stimulated slices. Our findings reveal a paradoxical microglial inactivation as a previously unrecognised feature of cryptococcal neuropathogenesis and suggest that immune silencing within the brain may contribute to fungal persistence and disease progression.

100 – Cryptococcal aggregation in the perivascular compartment drives glymphatic disruption during murine cryptococcal meningitis

Simran Atulkumar Patel

Yanga Bridget Pato^1,2,3, Anja de Lange^5, Mike Reiche^5, Caron Jacobs^5, Darren Thomson^4, and Rachael Dangarembizi^1,2,3

1. Division of Physiological Sciences, Department of Human Biology, Faculty of Health Sciences, University of Cape Town; 2. Neuroscience Institute, Faculty of Health Sciences, University of Cape Town, Groote Schuur Hospital, Cape Town, South Africa; 3. CMM AFRICA Medical Mycology Research Unit, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, South Africa

Cryptococcal meningitis (CM) is an invasive fungal infection of the brain associated with severe neurological complications including hydrocephalus and elevated intracranial pressure (ICP). Dilated perivascular spaces (PVS) have long been recognised as a radiological hallmark of CM but mechanisms underlying this pathology are poorly defined. Here, we hypothesise that neuropathology in CM may arise from glymphatic disruption and investigate cryptococcal distribution and its effect on glymphatic flow. Twenty-four C57BL/6 mice were intravenously injected with 5x10^4 Cryptococcus neoformans (FD651), Cryptococcus gattii (R265), or saline. To measure the distribution of fungal cells relative to the vascular network, optically cleared whole brains were imaged using light-sheet microscopy, followed by quantitative distance mapping to determine fungal proximity to blood vessels. Glymphatic influx was evaluated by intracisternal infusion of 10 uL of a fluorescent tracer (Alexa 555-ov albumin, 45 kDa) at 2 uL/min. Tracer penetration was determined by measuring the mean fluorescence intensity and thresholded area fraction across coronal sections collected from bregma +2 to -2. Three-dimensional spatial analysis revealed preferential aggregation of cryptococcal cells within PVS. Fungal aggregation was associated with disruption of glymphatic influx, as evidenced by reduced tracer penetration in infected mice compared to healthy controls. Furthermore, infected mice exhibited increased resistance to tracer infusion, reflected by the prolonged infusion times. Our findings provide the first experimental evidence of glymphatic disruption during CM, potentially arising from occlusion of the PVS by aggregated cryptococcal cells. This mechanism offers a plausible biological framework linking fungal neuroinvasion to the hydrocephalus and elevated ICP that characterises CM.

101 – Use of an immortalized human microglia cell line tostudy the unique recognition of Cryptococcus neoformans by human microglia.

Robbi Ross

Kassandra Arias-Parbul, Rebeca Candescu, Felipe H. Santiago-Tirado

University of Notre Dame

In immunocompromised individuals, Cryptococcus neoformans escapes from the lungs and disseminates to the central nervous system (CNS), where it causes an estimated 147,000 deaths annually. In the brain, C. neoformans interacts with microglia, the tissue-resident macrophages of the CNS. Previous studies showed that microglia are ineffective at controlling this fungal infection through mechanisms that remain unclear. Thus, I aim to understand the recognition and intracellular survival of C. neoformans by immortalized human microglia. I show that microglia have limited phagocytic activity that is specific to C. neoformans and independent of various cryptococcal factors. I also show that microglia respond to C. neoformans differently than other tissue-resident macrophages, especially relating to recognition, phagocytosis, and phagosome maturation. I have screened 2,800 cryptococcal mutants and identified novel genes, most of which are currently uncharacterized, that may be resp onsible for the yeasts ability to specifically evade phagocytosis by microglia. Interestingly, we find that factors such as cell size and chitin/chitosan exposure seem to be instrumental in yeast phagocytosis by microglia. Additionally, I show that microglia are ineffective at killing phagocytosed C. neoformans, and that this could be due to the ability of this yeast to disrupt phagosome maturation and induce phagosome membrane damage in these cells, which is partially dependent on fungal opsonization. These findings provide us with fundamental knowledge regarding cryptococcal pathogenesis in the CNS, specifically insight into how C. neoformans is recognized by microglia under different conditions, and how the yeast could be surviving and replicating within the microglial phagosome after engulfment.

102 – Ebselen leads Cryptococcus gattii yeasts to death by disturbing redox homeostasis and shows antifungal efficacy in a murine model of cryptococcosis

Leticia Serafim da Costa

Daniel Felipe Freitas de Jesus, Andriéli Bacega, Cristina de Castro Spadari, Kelly Ishida

Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, Brazil

Cryptococcus gattii is a World Health Organization priority fungal pathogen responsible for pulmonary and cerebral cryptococcosis in immunocompetent individuals, with limited therapeutic options. This study evaluated the antifungal activity of the organoselenium compound ebselen (EbSe) against C. gattii using in vitro assays and a murine model of cryptococcosis. Minimum inhibitory concentrations (MICs) were determined by broth microdilution for strain R265, clinical isolates (n=8), and distinct morphotypes, including enlarged capsule and titan cells, ranging from 0.52 g/mL. Time-kill assays demonstrated fast fungicidal activity within the first hours of exposure. EbSe reduced capsule size, increased capsular permeability, and inhibited melanization in L-DOPAsupplemented medium. Cell death protocols revealed DNA fragmentation, chromatin condensation, mitochondrial membrane depolarization, and membrane permeability after treatment. EbSe exposure increased reactive oxy gen species and lipid peroxidation, while reducing intracellular glutathione and total thiol levels. An increase in superoxide dismutase activity was also observed, suggesting a compensatory response to oxidative stress. Together, these findings indicate that EbSe disrupts fungal redox homeostasis, leading to oxidative damage and cell death. In a murine model, BALB/c mice were infected intranasally with 5x105 UFC/mice of C. gattii R265 and treated daily with Amphotericin (AMB, 1 mg/kg), Fluconazole (FLC, 100 mg/kg), EbSe (10 mg/kg) or combinations (AMB and EbSe; FLC and EbSe) for 6 or 13 days. EbSe treatment alone or in combination was associated with reduced fungal burden, particularly in the brain, highlighting its pharmacological activity during infection and supporting further investigation of its antifungal effects.

103 – A novel CpG-adjuvanted heat-killed Cryptococcus vaccine induces superior immune protection in an intramuscular model

Yu Zhang

Yina Wang1, Keyi Wang2, Amariliz Rivera2, Chaoyang Xue1

Rutgers University

Vaccines have been transformative in controlling infectious diseases. However, despite the rising global burden of invasive mycoses, no fungal vaccine is available for clinical use. Hence, there is a great unmet need to develop vaccines against fungal infections, including cryptococcosis. We previously developed a whole-cell vaccine candidate based on heat-killed C. neoformans fbp1 mutant cells (HK-fbp1), which provided complete protection against cryptococcosis following intranasal immunization by inducing durable pulmonary immunity. In this study, we developed a highly effective intramuscular and subcutaneous vaccine (HK-fbp1/ODN) by incorporating a CpG adjuvant into HK-fbp1. The adjuvanted formulation conferred robust protection against virulent C. neoformans challenge, achieving near-sterilizing immunity in multiple mouse strains. Immunological analysis revealed that intramuscular HK-fbp1/ODN vaccination promoted early recruitment of monocytes, monocyte-derived dendri tic cells, and neutrophils to the lung, accompanied by strong CD4+ T cell activation. Vaccine protection was associated with a spatially organized T cell response, characterized by proliferative priming in muscle-draining lymph nodes and potent Th1/Th17 effector cytokine production in lung-draining lymph nodes. IFN-ɣ was essential for vaccine-mediated protection, whereas other interferons were dispensable. Notably, HK-fbp1/ODN vaccination remained protective in mice lacking CD4+ T cells or B cells, underscoring its potential utility in immunocompromised hosts. Collectively, this study established a CpG-adjuvanted whole-cell cryptococcal vaccine that elicits coordinated innate and T cell-mediated immunity and is compatible with standard parenteral delivery, providing a strong foundation for the development of clinically relevant antifungal vaccines.

104 – Pharmacokinetics of low-dose flucytosine in patients with HIV-associated cryptococcal meningitis

Tiffany Chang

Morris K Rutakingirwa (b), Caleb P Skipper (c), Katharine E Stott (d), David B Meya (b,c), David R Boulware (c), Thomas C McHale (c), Lisa Coles (a), Sílvia M Ilamola (a), Melanie R Nicol (a)

University of Minnesota, College of Pharmacy, Experimental and Clinical Pharmacology Dept.

Background/Aims HIV-associated cryptococcal meningitis has a high case-fatality rate. A phase 2 trial (FLOOR) in Uganda demonstrated suboptimal efficacy of low-dose flucytosine (5-FC; 60 mg/kg/day in three divided doses for 10 days) based on cerebrospinal fluid (CSF) fungal clearance. Here, we characterize FLOOR plasma and CSF pharmacokinetics (PK) of 5-FC and examine associations between CSF exposure and clinical outcomes. Methods Plasma was intensively sampled 1, 2, 3-5 or 5-7, and 8 hours post-dose on treatment day 5. CSF was sparsely sampled on days 1, 3, 5-7, and 10-14. Plasma and CSF 5-FC concentrations were quantified using liquid chromatographymass spectrometry. Initial plasma PK parameters were estimated via non-compartmental analysis (NCA) using Phoenix Winnonlin 8.5.2.4. Fishers Exact Test using R 4.5.2 compared week 18 CSF sterility and rehospitalization and/or death between participants above/below the MIC90 threshold. Results Forty-eight participants (22 male, 26 female) contributed sparse sampling; eleven contributed intensive sampling. Median (IQR) steady-state plasma concentrations were Cmin 12.9 mg/L (9.66 - 30.2), Cmax 28.3 mg/L (14.6 - 36.1), and AUCtau 151.42 mg h/L (91.18 - 250.63). Seven participants had at least one CSF concentration below MIC90 and were less likely to achieve CSF sterility and more likely to experience rehospitalization or death; differences were not statistically significant (50% vs. 61% and 57% vs. 43%, respectively; p>0.6). Conclusions Transient CSF concentrations below MIC90 may be linked to poorer fungal clearance. NCA-derived PK estimates will be tested in a population PK model under development to define population exposures and their relationships to clinical outcomes.

105 – MMV1593537 as a Novel Antifungal Against Cryptococcus

Haroldo Cesar de Oliveira

Cssia Milena de Souza (1), Lvia Tavares Consolli (2), Michelli Alves da Silva (2), Rafael F. Castelli (1), Flvia G. Reis (1), Suelen Andria Rossi (1), Carlos Taborda (3), Marlon Dias Mariano dos Santos (1) and Marcio Loureno Rodrigues (1)

Department of Microbiology, Immunology, and Parasitology, Discipline of Cellular Biology, Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil.

Cryptococcosis, caused by Cryptococcus neoformans and C. deuterogattii, is a life-threatening systemic mycosis associated with high mortality and healthcare costs. Current antifungal therapies are limited by toxicity, suboptimal efficacy, and emerging resistance, highlighting the need for new treatments. This study aimed to characterize the antifungal candidate MMV1593537. MMV1593537 exhibited potent antifungal activity against C. neoformans (H99) and C. deuterogattii (R265), with a minimum inhibitory concentration (MIC) of 5 M, displaying fungicidal and anti-biofilm activities. The compound showed broad-spectrum activity against Candidozyma auris, Candida spp., Aspergillus fumigatus, Histoplasma capsulatum, Paracoccidioides spp., and Sporothrix spp., with MIC values ranging from 5 to 10 M. Treatment increased the detection of cell wall chitooligomers in Cryptococcus, C. auris, and Sporothrix, suggesting enhanced chitin hydrolysis. Accordingly, peaks in surface chitinas e activity were observed following exposure. Transmission electron microscopy revealed significant cell wall damage, including reduced thickness and marked cellular alterations. Proteomic analysis showed extensive remodeling. In C. neoformans, 29 proteins were exclusive to untreated cells and 66 to treated cells; in C. deuterogattii, 40 and 101 proteins were exclusive to control and treated conditions, respectively. Untreated cells of both species exhibited enrichment of protein interaction networks related to RNA metabolism, suggesting that MMV1593537 disrupts this pathway. Overall, MMV1593537 is a promising antifungal candidate, combining strong in vitro efficacy with low toxicity. Its mechanism appears to involve disruption of RNA metabolism and cell wall integrity, supporting further development as a therapeutic alternative for cryptococcosis.

106 – The role of thermotolerance and nutrient availability on resistance to fluconazole in clinical isolates of Cryptococcus neoformans.

Jessica Keeran

Lukasz Kozubowski

Eukaryotic Pathogens Innovation Center, Clemson University, Clemson, South Carolina, USA

Cryptococcus neoformans is the major causative agent of cryptococcosis and fungal meningoencephalitis across the globe and accounts for approximately 15% of all AIDS related deaths. One of the most applied antifungal medications for maintenance therapy in cryptococcosis is fluconazole (FLC). Unfortunately, development of resistance to FLC occurs frequently due to its fungistatic vs. fungicidal effect. The effects of environmental conditions on resistance to the drug fluconazole have been modeled quantitatively using Candida albicans, another common fungal pathogen. However, minimal research has been done to quantify and model the effects of environmental conditions on FLC drug resistance in a variety of C. neoformans clinical isolates. This study uses quantitative techniques that were previously utilized for C. albicans to characterize how environmental factors, including temperature and nutrient availability, impact FLC resistance in C. neoformans. Disk diffusion assays, i mage analysis software, diskImageR, and heat gradient assays were used to assess key differences in resistance to fluconazole between clinical isolates. Our results showed that resistance to fluconazole and responses to temperature and nutrient availability were strain specific and elucidates genetic factors as being a key influence on those responses. Our results also show distinct differences in thermotolerance and resistance to FLC between C. albicans and C. neoformans. Overall, this research displays the complex nature of antifungal drug responses in C. neoformans. Our results suggest that there could be clinical importance in distinguishing C. neoformans strains when treating cryptococcosis and could provide insights to help improve anticryptococcal therapy.

107 – Using MLST to explore relationships between environmental and animal associated isolates of Cryptococcus in a koala population.

Hannah Newton

Dr Luisa Monterio de Miranda (1), Dr Valentina Mella (1), Dr Astrid Van Aggelen (2), Prof. Mark Krockenberger (1)

Sydney School of Veterinary Science, The University of Sydney, Sydney, Australia

Understanding of Host-Pathogen-Environment interactions (HPEIs) during early cryptococcosis pathogenesis is lacking compared to late pathogenesis. Animals like the koala are excellent sentinels to enhance these knowledge gaps. Their restricted spatial movements, intimate relationships with their environment and high cryptococcal prevalence, help overcome key barriers to studying early pathogenesis in humans. This study aims to investigate the relationship between environmental and animal isolates collected from different origins in the HPEI framework in a captive koala population with a recent cryptococcosis outbreak. Ten individually housed koalas were screened. Six C. neoformans and 101 C. gattii spp. complex isolates (n=107) were collected from positive specimens for MLST. These consisted of 52 nasal isolates from eight koalas, 12 lesion isolates from two clinical cases and 43 environmental isolates from nine enclosures. Limited diversity was observed, with only six sequence types (ST) identified. Five STs were identified from nasal cavity specimens, with over half of the koalas (6/8) having two STs in their nasal cavities. Interestingly, one disease STs and three nasal STs were not detected in the environment. Only one ST was identified across all origin types. These results suggest acquisition of Cryptococcus from the environment and early pathogenesis is multifactorial and not simply a reflection of highly abundant STs. Further investigations using WGS and/or virulence assays are needed to provide a more wholistic picture of isolate diversity during different HPEIs. This study also supports use of sentinels like the koala as natural infection models to enhance understanding of cryptococcosis pathogenesis more broadly.

108 – Dormant Cryptococcus neoformans yeasts persist in a concealed state in a murine model of infection

Anamelia Lorenzetti Bocca

Rafaela L.L. Souza1,2., Gabriélly Bindo Trindade1,2., Vitória Bassi2., José Barcello Neto2., Raffael Jûnio, Araújo de Castro3., Clara Luna Freitas Marina3., Alexandre Alanio4, João Santana da Silva1,2.,

Bi-Institutional Translational Medicine, Oswaldo Cruz Foundation, São Paulo, Brazil and Center of Biotechnology, University of Brasília, Brasília, Brazil.

Several microorganisms, including Cryptococcus neoformans, can enter a dormant state characterized by reduced metabolic activity, transcription, and translation, enabling survival under unfavorable conditions with the potential to resume growth when environmental cues become permissive. The present study aimed to identify host immune factors that prevent or promote reactivation of dormant C. neoformans. We established an in vitro protocol to induce dormancy and generate viable but non-culturable (VBNC) yeasts and developed a murine model of latent infection using these cells. Wild-type (WT) mice infected with VBNC yeasts exhibited prolonged survival, and no fungal growth was detected in pulmonary tissue by colony-forming unit assays. However, following immunosuppression, high fungal burdens were recovered from the lungs and brains, confirming the presence of viable latent infection that did not cause mortality. In contrast, TLR-4-, TLR9-deficient mice and bone marrow-derived macrophages from these animals showed enhanced reactivation of dormant fungi compared to WT controls, even in the absence of immunosuppression. Moreover, at 110 days post-infection with VBNC cells, TLR9-deficient mice displayed reduced survival, indicating a role for TLR9 in maintaining fungal dormancy. Flow cytometric analyses further demonstrated an increased frequency of monocytederived dendritic cells in TLR9-deficient animals infected with dormant yeasts, implicating this population in the regulation of latency. Collectively, these findings indicate that the murine immune response, particularly TLR9-dependent pathways, contributes to maintaining C. neoformans dormancy and restricting fungal reactivation, thereby limiting infection-associated pathology.

SESSION 3

109 – Low CSF glucose is associated with increased CNS inflammation in adult patients with HIV-associated cryptococcal meningitis: a prospective cohort study.

Julia Palmucci

Ananta S. Bangdiwala (2), Abduljewad Wele (2), Samuel Okurut (3), Asmus Tukundane (3), Wilber Bakka (3), Suzan Mulwana (3), Mable Kabahubya (3), Jane Francis Ndyetukira (3), Olivie C Namuju (3), Morris K Rutakingirwa (3), Jane Gakuru (3), Timothy Mugabi (3), Sarah Kimuda (3), Enos Kigozi (3), Abdu K Musibire (3), Mahsa Abassi (4), David Meya (3), and David R Boulware (4)

Center for Global Health and Social Responsibility, University of Minnesota, Minneapolis, MN, USA

Background Low cerebrospinal fluid (CSF) glucose is a prognostic marker in several meningitis etiologies including tuberculosis, but its role in HIV-associated cryptococcal meningitis is less defined. We investigated if CSF glucose is associated with cryptococcal outcomes. Methods We conducted a prospective cohort study of 1,103 adults with HIV-associated cryptococcal meningitis in Uganda from January 2019 to May 2025. We grouped participants by CSF glucose into tertiles: low <45 mg/dL n=311, medium 45-74 mg/dL n=414, and high >75 mg/dL n=361. We assessed for baseline associations with CSF parameters and outcomes. Results Participants with low CSF glucose were more likely to present with altered mental status (GCS<15; P=0.001), higher CSF white cells (P<0.0001), and higher CSF protein (P<0.0001). Sixty percent (60%) of participants with low glucose had CSF white cell counts >5, compared to 45% and 28% among those with medium and high glucose, respectively (P<0.001). Flow cytometry (n=189) revealed that the low CSF glucose group had 4-fold more CSF CD4 cells (x=624 vs 160 cells/mL, P=0.024), more PD-1+ CD4 exhaustion (67% vs 49%, P<0.001), and lower monocyte activation (HLA-DR+, CD163+) compared to CSF glucose >45 mg/dL. CSF glucose showed a weak inverse correlation with baseline fungal burden (r=-0.10; P=0.0016), but glucose was not associated with in-hospital mortality, CSF early fungicidal activity, or CSF sterility. Conclusion Low CSF glucose was associated with severe clinical disease and CNS inflammatory response. A variety of mechanisms likely contribute to low CSF glucose, including increased metabolic activity in the brain due to infiltrating leukocytes and microbes.

110 – 15 years on: how the changing HIV treatment landscape has impacted cryptococcal meningitis rates in Cape Town

Kyla Murphy

Teagan Van Wyk (1,2), Kyla Murphy (2,3,4), Rhys Wenlock (5), Caraline Young (6), Richard Halley-Stott, (1, 7), Graeme Meintjes (1,3,8), Jonathan Naude

Department of Medicine, University of Cape Town, Cape Town, South Africa

Background: Cryptococcal meningitis (CM) is a life-threatening opportunistic infection, typically associated with advanced HIV. This study compares the epidemiology of CM in a high HIV-prevalence setting in South Africa with a study in a similar population 15 years ago. Methods: Retrospective clinical and laboratory information was collected for all adults who underwent a lumbar puncture at Mitchells Plain District Hospital between January 2022 and December 2023. Clinical episodes of CM were identified and analysed using descriptive statistics. Results were compared to a 2010 cross-sectional analysis by Jarvis et. al in a geographically-similar population. Results: 2022 clinical presentations were captured. 262 episodes of microbiologically-confirmed meningitis were recorded of which CM accounted for 34% (88/262); compared to 63% in Jarvis et. al (2010). Most CM episodes (95%) were confirmed by CrAg LFA compared to only 40% in 2010. In this study, 99% (87/88) of CM cases were HIV-positive. Although 84% were ART-experienced, only 23% (19/83) were reportedly adherent to ART at presentation. CM mortality was 37% (30/87) overall. Although reflex serum CrAg screening was in place, only 39% (34/88) had a serum CrAg result prior to admission of which 68% (23/34) were serum CrAg positive. Conclusion: CM occurred predominantly in patients with advanced HIV with a median CD4 count of 34 cells/uL. While prior ART-exposure in CM was high, most patients had interrupted ART prior to CM. In comparison to the Jarvis et al. (2010) cohort, availability of CrAg CSF testing has improved with almost all CM cases confirmed with CrAg LFA.

111 – Delayed presentation to care among Ugandan adults with HIV-associated cryptococcal meningitis: a prospective cohort study

Mable Kabahubya

Richard Kwizera (1), Abduljewad Wele (2), Biyue Dai (2), Alisat Sadiq (1), Suzan Mulwana (1), Jane Frances Ndyetukira (1), Enock Kagimu (1), Laura Nsangi (1), Tessa Adzemovic (1), Jayne Ellis (1,3), David R Boulware (4), David B Meya (1,4, 5), Radha Rajasingham (4)

Department of Research, Infectious Diseases Institute, College of Health Sciences, Makerere University, P.O. Box 22418, Kampala, Uganda.

Background: Mortality from cryptococcal meningitis remains high between 10%-25% at two weeks within clinical trials. We sought to determine whether participants with cryptococcal meningitis who present late to care have worse clinical outcomes compared with participants who do not present late to care. Methods: We analyzed data from a cohort of hospitalized adults with HIV-associated cryptococcal meningitis. Delayed presentation to care was defined as having a headache for >21 days at the time of hospitalization. We used logistic regression to investigate the association with delayed care-seeking, and its effect on length of hospital stay. Cox proportional hazards ratio was conducted to assess the association between delayed care seeking and in-hospital mortality. Results: Among 1,032 participants with cryptococcal meningitis, the median CD4 cell count was 28 cells/L and the median duration of headache prior to hospitalization was 14 days (IQR: 7-29). Overall, 26% of part icipants presented to hospital with >21 days of headache. When comparing those with >21 days of headache versus those with <21 days of headache, participants who were on antiretroviral therapy were more likely to delay seeking care (AOR= 1.43, p-value= 0.016). There was no significant difference in hospital duration (p=0.329) and two-week mortality (p=0.088) between those who presented late to care versus those who did not. Conclusions: Late presentation to care is common, with 26% of participants presenting with >21 days of headache. There was no difference in two-week mortality and length of hospitalization between participants with >21 days of headache versus <21 days of headache.

112 – Antifungal susceptibility profile of Cryptococcus spp. isolated from patients with neurocryptococcosis in Brazil.

Nalu Teixeira de Aguiar Peres

Bruna C. T. Almeida1, Ludmila Gouveia-Eufrásio1, Danielle L. da Silva1, Lislaina S. Corrêa1, Rafael W. Bastos2, Luana Rossato3, Livia Kmetzsch4, Daniel A. Santos1,5, Nalu T. A. Peres1,5.

Federal University of Minas Gerais

Cryptococcosis is frequently associated with meningoencephalitis in immunocompromised individuals and high mortality. Due to the limited availability of treatment and increasing clinical resistance, understanding the susceptibility profile of circulating strains is essential. The aim of this work was to evaluate the susceptibility profile of Cryptococcus isolated from patients in five Brazilian States. A total of 252 isolates were evaluated, 193 identified as C. neoformans and 59 as C. gattii. Susceptibility testing revealed intermediate resistance to flucytosine (5-FC) in two isolates, and resistance to fluconazole (FCZ) in six isolates. MIC evaluated from sequential samples from the same patient showed differences in susceptibility, suggesting a transient resistance or coinfection with different strains. Three C. gattii isolates showed simultaneous resistance to 5-FC and FCZ. For amphotericin B, no elevated MIC values were observed. Exposure of a susceptible isolate to 5- FC induced the expression of FCY2, responsible for drug internalization, while FCY1 and FUR1, involved in 5-FC intracellular activation, were downregulated. FCZ exposure upregulated the efflux pump genes AFR1 and MDR1, whereas ERG11, involved in ergosterol synthesis, remained highly expressed, possibly as a compensatory mechanism to maintain ergosterol production. Two resistant isolates showed high expression of AFR1 and MDR1, in the absence of drugs. These findings indicate that surveillance of resistance is essential to evaluate treatment of cryptococcosis and clinical susceptibility testing should be monitored during treatment to detect a resistance phenotype. Further studies will provide insights into the differences in sequential isolates from the same patient and the underlying resistance mechanisms.

113 – Disseminated HIV-Associated Cryptococcosis with Pulmonary Embolism in Pregnancy: A case report

Mulungi Jonathan

Jane Gakuru2, Kezia Vecchiet3, Suzan Namombwe2, Faizo Ssekindi2, Joseph Baruch Baluku1, Felix Bongomin4,5, Sarah Nakubulwa7, Oliver A. Cornely6, David B. Meya2,7

Infectious Diseases Institute Makerere University

Case Presentation Cryptococcal meningitis (CM) is a leading cause of mortality in pregnant patients with Advanced HIV disease. Despite the updated WHO treatment guidelines for CM following the AMBITION trial, there is still a gap in treatment of CM in special populations like pregnant women due to the well-established teratogenic effects antifungal medication. We present a detailed case of a newly diagnosed HIV-1 pregnant patient with cryptococcal meningitis, pulmonary embolism. A 28-year-old female, Gravida 2 para1+0 at 27 weeks of gestation with advanced HIV disease (CD4 count of 5 cells/L). She was hospitalized with concerns of a persistent headache associated with neck stiffness. She also reported history of difficulty in breathing and low-grade fevers. She complained of generalized body weakness and an ulcer in the left axilla. The patient reported to have attended antenatal care once during the current pregnancy. She was in an HIV sero-discordant relationship. Discussion The management of CM in pregnancy is complex as there are no standard treatment guidelines. Patient was treated using intravenous liposomal amphotericin B at 3 mg/kg for 10 days was instituted. The Lumbar Puncture was performed had an opening pressure of 170mmH20 and the CSF analysis confirmed the presence of 3,000 Colony Forming Units (CFUs). On Day 7, the culture results revealed no growth. Baseline CD4 count was 5 cells/ L, Despite adequate therapy, the patient died. An autopsy revealed a mildly enlarged liver, congested right lung, gallstones. Histopathology revealed focal cystic areas in the kidneys containing blastoconidia of Cryptococcus

114 – Evolution of Laboratory Diagnostics for Cryptococcosis and Missing Links to Optimize Diagnosis and Outcomes in Resource-constrained Settings

Richard Kwizera

Tadeo K Kiiza (1), Andrew Akampurira (1,2), Sarah Kimuda (1), Timothy Mugabi (1), David B Meya (1,3)

Infectious Diseases Institute, College of Health Sciences, Makerere University, P.O. Box 22418, Kampala, Uganda.

Background and aims: Early diagnosis of Cryptococcal meningitis (CM) is crucial in improving treatment outcomes. Despite advances and the availability of modern and point-of-care diagnostics for cryptococcosis, gaps still exist in resource-constrained settings (RCS), leading to unfavourable treatment outcomes. We aimed to review the current outstanding issues or missing links that need to be filled to optimise the diagnosis of cryptococcosis in RCS to improve treatment outcomes. Methods: We highlight the missing links to optimise diagnosis and outcomes, including some practical recommendations. Results: The major missing links to optimise treatment outcomes among CM patients involve troubleshooting the limitations of fungal culture, addressing delayed diagnosis, developing more versatile diagnostics and improving access to the CrAg LFA, antifungal susceptibility testing and therapeutic drug monitoring. However, from RCS, we find that most facilities have a light microscope since it is still an essential tool in diagnosis. Trained human resource capacity in the identification of cryptococcal meningitis is still key. Procurement issues also affect the availability of tests, unless RCS are funded by outside sources. There remain issues of inequality and equity of resource allocation between the private vs government-run laboratories. The high cost of tests is still a limiting factor to access in RCS. Implementation of these tests in the RCS is also hindered by competing diseases and health systems of these settings, which in turn affect the diagnosis of CM. Conclusions: Major issues still exist in the diagnosis of cryptococcosis in RCS. Addressing them could help to improve treatment outcomes.

115 – Identification of synergistic compounds in combination with fluconazole from the Prestwick Chemical Library against Cryptococcus neoformans.

Alba Torres Cano

Alba Torres-Cano1, Alejandra Lora-Plaza1, Óscar Zaragoza1,2

Instituto de Salud Carlos III, Madrid, Spain

The treatment of Cryptococcus neoformans is limited due to its low susceptibility to fluconazole, compared to other fungal species, and intrinsic resistance to echinocandins, resulting in a restricted range of therapeutic options. In this context, drug repurposing emerges as a promising alternative strategy, as it consists of identifying new biological effects of drugs already used for other purposes, offering significant advantages in terms of cost, time, and safety compared to de novo drug development. In this study, a total of 1,520 off-patent, FDA-approved compounds from the Prestwick Chemical Library were screened to identify agents with synergistic activity in combination with fluconazole against C. neoformans. An initial high-throughput screening allowed the selection of 12 promising compounds, including acetylsalicylic acid, pinaverium bromide, thioridazine hydrochloride, cianidol, risedronic acid monohydrate, tegaserod maleate, alendronate sodium, ibufenac, flurbiprofen axetil, oxiglutatione, atorvastatin, and avermectin B1. Synergistic interactions were subsequently confirmed by checkerboard assays using six C. neoformans clinical isolates. Synergy with fluconazole was validated for pinaverium bromide, thioridazine hydrochloride, alendronate sodium, oxiglutatione, ibufenac, atorvastatin, avermectin B1, tegaserod maleate, and risedronic acid monohydrate. To further assess the translational potential of these combinations, cytotoxicity was evaluated in RAW 264.7 macrophages, revealing toxicity at the highest tested concentrations of pinaverium bromide, tegaserod maleate, and thioridazine hydrochloride. In addition, in vivo safety assessment using Caenorhabditis elegans is currently being conducted to further characterize compound efficacy. Overall, these results highlight drug repurposing as a valuable approach for identifying novel antifungal combinations against C. neoformans.

116 – CMV viremia is associated with impaired CSF fungal clearance amongst 509 Ugandan adults with HIV-associated cryptococcal meningitis

Timothy Mugabi

Kathy Huppler Hullsiek3, Mark Blackstad1, Laura Nsangi2, Jayne Ellis4, David B Meya1,2, Mark R Schleiss1, David R Boulware1, and Caleb P Skipper on behalf of the IDI Meningitis Research Team

Infectious Diseases Institute, Makerere University, Kampala, Uganda

Background: Cryptococcal meningitis is the leading cause of HIV-associated meningitis with 10-week mortality of 25-30%. Cytomegalovirus (CMV) viremia occurs in 30-50% of adults with advanced HIV disease and is associated with increased mortality in cryptococcal meningitis through unclear mechanisms. We aimed to determine whether CMV viremia impairs CSF fungal clearance. Methods: We measured CMV DNA levels by quantitative PCR in baseline cryopreserved plasma from Ugandans with cryptococcal meningitis enrolled in clinical from 20102024. Amphotericin-based induction therapy evolved over time according to international standards. Early fungicidal activity (EFA) was calculated as the negative slope change in quantitative CSF fungal burden (log_{10} CFU/mL/day), estimated by linear regression. We analyzed odds of achieving sterility and all-cause mortality through 18 weeks. Results: Of 509 Ugandan participants with EFA available, we found CMV viremia in 37% (187/509). CMV viremia was significantly associated with lower CD4 counts, lower hemoglobin, fewer CSF white cells, and higher baseline fungal burden ($p<0.01$ for all). Participants with CMV viremia (median EFA=0.31 [IQR, 0.170.44]) were less likely to sterilize CSF by Day 18 after antifungal therapy (36% vs 54%; $p<0.001$) as compared to participants without CMV viremia (median EFA=0.35 [IQR, 0.220.51]). The combination of CMV viremia and EFA<0.20 dramatically reduced the odds of achieving Day 18 CSF sterility (OR=0.01 [95%CI, 0.000.05]). Participants with CMV viremia had higher mortality at 18 weeks (43% vs 26%; $p<0.001$). Discussion: CMV viremia is associated with impaired CSF fungal clearance, providing a possible mechanism for why CMV viremia is associated with excess mortality in cryptococcosis.

117 – Incidence of cryptococcosis over a decade period in a tertiary care hospital and Fungal Clinic in northern India.

Jagdish Chander

R P S Punia2, Deepak Aggarwal

Department of Microbiology1, Government Medical College Hospital, Chandigarh & Fungal Clinic4, Panchkula

Background and Aims: Worldwide, cryptococcosis has shown a rising trend, largely driven by the ongoing HIV/AIDS pandemic and increasing numbers of immunocompromised individuals. Although India carries a substantial burden due to HIV-associated infections, the prevalence remains lower than that reported in African countries. Northern India, characterized by relatively low HIV prevalence compared to southern regions, correspondingly reports relatively fewer cases of cryptococcosis. This study was undertaken to analyse the incidence and antifungal susceptibility patterns of cryptococcal isolates over a ten-year period (January 2016-December 2025) at a tertiary care centre and Fungal Clinic in North India.

Methods: Clinical specimens including cerebrospinal fluid (CSF), biopsy tissues and fine needle aspiration cytology (FNAC) samples were processed using standard microbiological and histopathological techniques. Microscopy included India ink preparation, H&E, PAS, and MGG staining. Fungal cultures were performed on blood agar, Sabouraud dextrose agar, and sunflower seed agar. Identification was based on colony morphology, biochemical tests, serotyping and confirmed using MALDI-TOF MS. Selected isolates were further validated at the reference laboratory.

Results: A total of 23 cases were diagnosed over ten years, predominantly in males (19/23), with most patients being HIV-positive (21/23). CSF latex agglutination was positive in 20 cases. The majority of isolates were Cryptococcus neoformans var. grubii (15), followed by var. neoformans (3), with one case each of C. gattii and C. diffluens. All isolates were sensitive to amphotericin B and fluconazole. Majority of isolates were recovered from CSF samples.
Conclusions: Cryptococcosis incidence remains relatively less common in northern India.

118 – Serum Cryptococcus antigen testing: Results of an external quality control program 2022 - 2024

Volker Rickerts

Silke Kappler (2), Patricia Kaiser (2) Ilka McCormick Smith (1)

Robert Koch-Institute, Berlin, Germany

Cryptococcus antigen (CRAG) testing is essential for cryptococcosis management. Predictive value of semiquantitative titers in persons living with AIDS has been documented. Accuracy of semiquantitative titers in routine testing is difficult to assess. We describe an external quality assessment (EQA) program for accuracy of semiquantitative CRAG testing in non-specialized laboratories. In Germany, laboratories that provide CRAG testing reimbursed from health insurances are required to participate in an EQA program organized by Instand e.V., a German, non-profit interdisciplinary scientific medical society providing EQA programs for medical laboratories. Test samples are prepared using antigen from culture supernatants of C. neoformans H99 spiked into serum. CRAG titers vary between 1:80-1:2560. They are controlled before and after shipment to participants. They receive a positive and negative sample. The acceptable range is derived from the participants mean plus/minus two dilutions. We summarize results of participants using the IMMY lateral flow assay (lfa), the most commonly used assay. Between 68 and 80 (median 73) laboratories participate in the program. Use of lfa increased from 41% to 60% of participants during the observation period. The Median of participants semiquantitative results were within 1-2 dilutions steps from results of the EQA provider irrespective of sample transport. Between 0 and 16% of participants (Median 5.4%) were outside the acceptable range (plus/minus 2 dilutions) in the assessments. Laboratories increasingly use lfa for CRAG testing. Semiquantitative results in routine testing may vary considerably, limiting the use as prediction tool for individual patients.

119 – Analyzing genomic and phenotypic variation within a large clinical Cryptococcus population to identify determinants of virulence and disease outcome

Raphael Brosula

Katharine Bowers, Poppy Sephton-Clark, Jacqueline Villanueva Govea, Dena Toffaletti, David S. Lawrence, Thomas S. Harrison, Joseph N. Jarvis, AMBITION Study Group, Jennifer Tenor, John Perfect, Christina Cuomo

Infectious Disease and Microbiome Program, The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA

Cryptococcus neoformans is the main pathogen responsible for cryptococcosis, a disease which results in an estimated 112,000 deaths per year. Pathogen-specific characteristics, such as an infectious isolates genetic background and expressed phenotypic traits, may influence poor patient outcomes. However, the relative contributions of genetic diversity and phenotypic variation to clinical outcomes remain poorly understood. Here, we leverage clinical, genomic, and in vitro phenotypic data from 590 C. neoformans isolates collected through the AMBITION clinical trial, spanning Uganda, Malawi, South Africa, Botswana, and Zimbabwe. As previously observed in other studies, we find that both fungal burden and the rate of fungal clearance correlate highly with mortality. Whole-genome sequencing and phylogenetic analysis reveal that, while we identify all four major lineages (VNI, VNII, VNBI, and VNBII), 87% of our isolates are within the VNI lineage. Genome-wide association study approaches identify variants linked to fungal burden, corroborating results from previous studies linking natural genetic variation to disease outcomes. In vitro phenotypic profiling of clinical isolates reveals heterogeneous growth in response to stressors which target key virulence traits, including cell wall stress, elevated temperature, and hypoxia. This study represents one of the largest genotypic and phenotypic characterizations of clinical C. neoformans isolates, providing a resource to complement previous and future studies of virulence within this pathogen. By analyzing both genomic and phenotypic diversity, we can illuminate biological mechanisms and pathways underlying pathogenicity, host adaptation, and clinical outcomes.

120 – Susceptibility and Genomic Testing of Cryptococcus gattii and Cryptococcus neoformans Clinical Isolates from Mozambique

Tshiama Miriam Mwamba

Tshiama M. Mwamba1,2, Serisha D. Naicker1,2, Aquino Nhantumbo3, Sergio Massora4, Ruth Mpembe1,2, Tsidiso G. Maphanga1,2, Rudzani C. Mashau1, Stanford Kwenda2, Nelesh P. Govender1,2

Wits Mycology Division, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Cryptococcus accounted for 38% of laboratory-confirmed meningitis cases in sentinel surveillance of adults with suspected meningitis in Mozambique (2016-2017). In 2019, fifteen isolates, cultured from adults with HIV-associated cryptococcal meningitis who had not responded to treatment, were referred to the National Institute for Communicable Diseases in South Africa. Isolates were first inoculated onto Niger seed agar and L-canavanine glycine bromothymol blue (CGB) agar and then identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. Antifungal susceptibility testing was performed using commercial broth microdilution (Sensititre) and gradient diffusion (Etest) methods, and minimum inhibitory concentrations were interpreted using Clinical and Laboratory Standards Institute epidemiological cut-off values for VNI or VGI. Whole genome sequencing was undertaken. Nine isolates (60%) were phenotypically identified as Cryptococcus neoformans species-complex and six (40%) as Cryptococcus gattii species-complex. All isolates were categorised as wild-type to azoles, flucytosine and amphotericin B. Fluconazole MICs ranged from 4-8 mg/L; 9/15 (60%) had an MIC of 8 mg/L. All C. neoformans isolates were molecular type VNI, while C. gattii isolates included one VGIV and five VGI. No known resistance-conferring mutations were detected. Despite reported treatment non-response in these clinical cases, all referred isolates had wild-type susceptibility phenotypes. However, fluconazole MICs of 8 mg/L in 60% of isolates could have resulted in suboptimal pharmacodynamic target attainment during periods when fluconazole daily dosing was <800 mg. Treatment non-response in cryptococcal meningitis may also be a consequence of other factors such as suboptimal induction therapy omitting flucytosine or inadequate pressure management.

121 – Neofunctionalization of H1 linker histones drives divergent gene expression and histone methylation in Cryptococcus neoformans

Grace J. Paul

Nicolas Helmstetter, Qinxi Ma, Diana Tamayo, Tal Goodisman, James W. Harrison, Elizabeth R. Ballou, Rhys A. Farrer.

University of Exeter

The opportunistic pathogen Cryptococcus neoformans adapts to diverse host microenvironments during infection, yet the contribution of epigenetic mechanisms remains unclear. Comparative genomics across 50 basidiomycete species revealed that one chromatin protein, the linker histone H1, is uniquely duplicated across the entire Cryptococcus genus, generating paralogs H1.51 and H1.52 with divergent evolutionary trajectories. Molecular evolution analyses revealed that H1.51 experienced intensified selection with episodic positive selection in clinical isolates, while H1.52 evolved under sustained purifying selection, indicating adaptive neofunctionalization. H1.52 deletion triggered transcriptional reprogramming of 1,561 genes (23% of the transcriptome), with downregulation of nucleolar function, ribosome biogenesis, and translation machinery, whereas H1.51 loss produced minimal transcriptional effects. We found that H1.52 governs chromatin architecture. Its deletion expanded H3K4me2 euchromatin coverage 1.54-fold (from 18.4% to 28.4% of the genome) into broader constitutive domains with loss of H3K9-marked heterochromatin islands. Following prolonged culture under host mimicking conditions (3-, 5-, and 7-day periods), H1.52 deletion resulted in enhanced metabolic activity and accelerated growth recovery compared to wild-type. These findings establish that Cryptococcus neoformans H1.52 coordinates chromatin compaction, maintains H3K9-marked heterochromatin islands and is a regulator of metabolic responses to host stress.

122 – ERG11 Mutations Reshape Fluconazole Binding in Cryptococcus

Lexia Henning

Alfred Botha(2) and Angela Botes(1)

School of Molecular and Cell Biology, University of the Witwatersrand, South Africa

Antifungal drug resistance (AFR) complicates the treatment of invasive fungal diseases (IFDs) such as cryptococcosis, particularly in immunocompromised individuals and those in resource-limited settings. Drug resistance often arises from single-nucleotide polymorphisms (SNPs) in genes encoding antifungal targets such as ERG11. This study assessed trends in cryptococcal AFR, identified resistance-associated SNPs and investigated their impact on protein folding and ultimately ligand interactions. Clinical and environmental cryptococcal isolates, originating from South Africa, were tested for antifungal susceptibility to fluconazole (FLC) using the microbroth dilution assay. The ERG11 gene of ten resistant and six susceptible isolates was sequenced and analysed for SNP identification and AFR correlation. Protein modelling, molecular docking and dynamic simulations assessed the impact of the SNPs corresponding protein mutations on lanosterol 14-demethylase structure, FLC and natural substrate interaction. Environmental isolates exhibited significantly higher AFR than clinical isolates ($p < 0.001$). Additionally, temporal and regional analyses revealed increased resistance in recent (post-2018) compared to older (pre-2010) isolates, with distinct spatial distributions ($p < 0.001$). Multiple ERG11 SNPs were identified, including South African-specific variants that alter the substrate-interacting residues and the haem motif. Molecular docking and dynamic simulations confirmed reduced FLC affinity and enhanced lanosterol binding in an extremely resistant variant (FLC minimum inhibitory concentration > 64 g/mL) compared to a susceptible variant. These findings highlight evolving AFR strategies, structural SNP impacts, emphasising the need for integrated surveillance and antifungal stewardship in high-risk settings.

123 – Gene content diversity in Cryptococcus neoformans

Blake Billmyre

Claire Reichardt

University of Georgia

Cryptococcus neoformans causes an estimated 147,000 deaths per year worldwide, primarily in immunocompromised patients. C. neoformans consists of 4 different molecular types: VNI, VNII, VNBI, and VNBII. While we understand sequence variation separating these groups, we have a limited understanding of the differences in genetic content that differentiate each isolate. We de novo assembled a pangenome using Illumina sequences of 384 diverse C. neoformans isolates. We curated a set of C. neoformans orthologs that are either core (present in >95% of genomes), accessory (present in >=95% and <1 genomes), or singletons (present in 1 genome). Most genetic content variation in C. neoformans is the result of variation in accessory gene content. Our pangenome revealed that a large portion of accessory genes were located near subtelomeres in the H99 reference genome. We also found numerous accessory genes that were limited to specific molecular types and that many singletons shared cl ose sequence similarity to closely related species of Cryptococcus, consistent with introgression or incomplete lineage sorting events. We are currently using transposon mutagenesis to explore the functional consequences of variation in gene content across the species.

124 – Identifying Cryptococcus spore-enriched proteins and their roles in spore coat restructuring during germination

Seth D. Greengo

Benjamin J. Chadwick, Joshua J. Coon, Christina M. Hull

Department of Biomolecular Chemistry, University of Wisconsin - Madison, Madison, WI, USA

To colonize new environments, including a mammalian lung, fungal spores must undergo germination into a vegetatively growing cell type. For Cryptococcus, this necessitates significant morphological changes in which a small, ovoid spore becomes larger and rounder as a yeast emerges laterally from the original spore structure. Functions such as spore coat degradation and new cell wall synthesis are likely crucial for spores to undergo the changes required for germination into yeast, yet little is known about the molecular pathways involved in these processes. To determine how protein identities and levels change during germination, we carried out a comprehensive analysis of the spore proteome during germination (at 6 time points over 10 hours). From this dataset, we identified 19 highly spore-enriched carbohydrate active enzymes (CAZymes) that we hypothesize are key players in spore coat restructuring during germination. Among these proteins are a chitin synthase, an endochit inase, an endo-1,3-beta glucanase, and a polysaccharide deacetylase, suggesting that cell wall modification plays a crucial role in the germination process. We are creating strains with targeted knockouts of the genes encoding these proteins to determine how their deletion impacts spore germination. We will evaluate the rates and efficiency of spore germination at both single-cell and population levels using quantitative germination assays (QGAs), and we will identify differences in spore carbohydrate levels and localization using fluorescence microscopy. These studies promise to reveal fundamental aspects of spore biology and identify mechanisms by which germination occurs, opening the door to more targets for prophylactic antigerminant therapies.

125 – From Cryptococcus neoformans in vivo gene expression to disease

Dena Toffaletti

Jaqueline Villanueva Govea (1), Daniel Chang (1), Oneeb Chaudhry (1), Charles D. Giamberardino (1), Joseph N. Jarvis (3), Christina A. Cuomo (4, 5), Jennifer L. Tenor (1), John R. Perfect (1,2)

Duke University School of Medicine, Dept. of Medicine, Division of Infectious Diseases

Hypothesis: Highly expressed Cryptococcus neoformans genes in human cerebrospinal fluid (CSF) contribute to cryptococcal meningoencephalitis pathogenesis. Methods: We examined 324 C. neoformans genes selected from the top 500 genes with the highest median transcript abundance in human CSF. Using site-directed deletion strains from a genome-wide deletion library, we assessed their roles in virulence-associated traits and stress responses. Results: In vitro phenotypic analysis identified 94 deletion strains with increased sensitivity to cellular stressors and/or inhibitors of key cellular processes. Eighteen strains exhibited heightened sensitivity to at least five conditions. These genes included candidates involved in putative arginine biosynthesis, mannoproteins, and proteins of unknown function. Uncharacterized genes were further analyzed through construction of deletion and wild-typerestored strains; paralogous genes were evaluated using double-deletion mutants to ass ess functional redundancy. Protein localization was also determined. Several single and double-deletion mutants displayed altered disease progression in a mouse model. Conclusion: These findings demonstrate that genes highly expressed in human CSF contribute to C. neoformans pathogenesis. Our results support the use of in vivo gene expression profiles as a powerful strategy for functional inference, particularly for genes of previously unknown function.

126 – Laccase Drives the Warburg Effect in Melanized Cryptococcus neoformans upon Glucose Depletion

Francisco Hernandez

Dr. Emma Camacho, Dr. Arturo Casadevall

Johns Hopkins School of Public Health

Cryptococcus neoformans is a WHO critical fungal priority pathogen as a result of antifungal resistance and high mortality rates of approximately 41-61% in people living with HIV. Melanin biosynthesis from exogenous phenolic substrates is an established stress response in Cryptococcus neoformans to confer resistance to oxidative damage, antifungal drugs, and nutrient starvation. Strikingly, we have identified that melanized cells exhibit a metabolic reprogramming characterized by lactate production and accelerated budding rate with lipid anabolism upon glucose deprivation. As the rate limiting step of melanization via its diphenol oxidase function, the laccase enzyme coordinates this metabolic switch by integrating signals from oxygen and glucose. In fact, laccase1 deletion exhibits drastic oxygen consumption rates compared to WT at low glucose conditions. RNAseq analysis reveals upregulation of dioxygenases in the laccase mutant along with severe defects in lipid and heme synthesis, which together contribute to compromised oxygen sensing. In this study, we characterize moonlighting functions of the laccase, including processing of the sterol regulatory element binding protein, to expand its critical role in establishing cryptococcal infection beyond melanin synthesis. Collectively, we have determined that the laccase drives glucose consumption rate through a Warburg-like shift to outcompete the host defenses of glucose during nutrient limitation. In macrophage coinfection, this alternate metabolism results in greater macrophage killing by melanin-coated yeast than no-melanin control, an evolutionary advantage offset with increasing glucose levels. These novel findings suggest that melanin is a metabolic adaptation to survive nutrient starvation and induce alternate biosynthetic pathways to overcome host immunity.

127 – Chitin as a Hidden Driver of Cryptococcus neoformans Virulence

IRERE HILAIRE

Liliane Mukaremera , Neil A.R. Gow

MRC CMM/ Exeter University

Meningitis caused by the fungal pathogen Cryptococcus neoformans remains a major global health problem, causing an estimated 152,000 cases and 112,000 deaths annually among people living with HIV/AIDS. The high mortality highlights limitations of current antifungal therapies and underscores the need to better understand fungal-traits that drive disease severity during infection. Cryptococcus is surrounded by a two-layered, sugar-rich structure: an outer polysaccharide capsule and an inner-cell wall. While the capsule is a well-established virulence factor, the contribution of cell wall components remains poorly defined. In this study, we screened 200 clinical C. neoformans isolates grown in physiologically relevant Human Plasma-Like Medium (HPLM) and selected 10 stable low-chitin and 10 stable high-chitin isolates representing the extremes of the chitin spectrum for in-depth analysis. Using these extreme phenotypes, we observed marked differences in fungal population struct ure and function. Low-chitin isolates were dominated by small-cell populations, exhibited higher metabolic activity, and were taken up more efficiently by macrophages. In contrast, high-chitin isolates were enriched for larger-yeast cells. Despite their smaller-cell bodies, low-chitin isolates produced proportionally larger capsules and achieved higher fungal burdens in murine lung infection models. Together, these findings indicate that cell wall chitin content is not only involved in mediating cell shape and environmental stress protection but also strongly influences Cryptococcus population structure and metabolic activity. The pronounced differences between high- and low-chitin isolates suggest that chitin acts as a dynamic regulator of key aspects of fungal biology. Further studies are underway to define the mechanism these phenotypes contribute to pathogenesis.

128 – Carbon starvation induces coincident capsule and cell wall remodeling in Cryptococcus neoformans

Elise Bedford

Leandro Buffoni Roque da Silva, Daniel Smith, Christopher W. J. Lee, Quigly Dragotakes, Arturo Casadevall, James W. Kronstad

University of British Columbia

The pathogenesis of Cryptococcus neoformans is largely attributed to the impact of the polysaccharide capsule on the survival of the fungus in the harsh conditions of the host. These conditions include a robust immune response and nutrient limitation in different tissues. Little is known about the survival mechanisms of C. neoformans in nutrient-deprived conditions, and a key unanswered question is whether the fungus can use capsule material for nutrition during conditions of carbon limitation. We addressed this question by measuring alterations in capsule and cell wall in response to carbon limitation and found an influence on capsule porosity and density without a change in diameter. RNA-seq analysis of the response to carbon limitation identified transcripts for enzymes with potential relevance to polysaccharide changes, including carbohydrate-active enzymes. Subsequently, the impact of a selected set of enzymes was evaluated with capsule and cell wall-relevant mutants l acking Cas1 and Cas3 (O-acetylation of capsule polysaccharide), Chs1-8 (chitin synthases), Cps1 (hyaluronic acid synthase), and Kre64 (β-glucan specific glycosidase). Overall, our findings show that C. neoformans responds to carbon starvation by increasing capsular and cell wall permeability through interactions between cell wall components (α- and β-glucans and chitin) and capsules that alter their density and porosity. The results also suggest that C. neoformans does not substantially degrade the capsule polysaccharide under the conditions of carbon limitation employed in this study.

129 – Unravelling kinetochore assembly principles of the critical fungal pathogen, C.neoformans

Vimal Das Edakkattu Kundil Sivadasan

Zhaoyue Yan, Anjitha Gireesh, Maria Alba Abad, Sreekrishna Bhat, Kevin Hardwick, Kaustuv Sanyal, A. Arockia Jeyaprakash

University of Edinburgh

Cryptococcus neoformans is a fungal pathogen known to cause meningitis in humans, particularly dangerous for immunocompromised individuals. An estimated 220,000 annual cases of Cryptococcal meningitis occur worldwide. Increasing resistance of the pathogen to antifungal drugs, due to the high genetic diversity of C. neoformans, is a growing concern. During human infections, many C. neoformans cells (~5m diameter haploid yeast form) undergo a striking morphological transition. They increase size to form thick-walled 10-100m diameter cells, called polyploid titan cells. Remarkably, through an unknown reductive division mechanism polyploid titan cells bud off viable haploid progeny. However, not much is known about how this division is achieved. Thus, knowledge of the chromosome segregation machinery is crucial to uncover therapeutic vulnerabilities of this pathogen. The kinetochore is a multi-subunit protein complex that is key for the segregation of the genetic material during cell division. Interestingly, a study by Sridhar et al., 2021 revealed that the kinetochore composition of C. neoformans is significantly divergent compared to canonical yeast and human systems, with a loss of the whole Constitutive Centromere Associated Network (CCAN) except for CENP-C and its replacement by a single novel kinetochore protein called Bridgin. Here we aim to understand the molecular mechanisms of accurate chromosome segregation in C. neoformans by recombinantly reconstituting the components of the C. neoformans kinetochore and structurally characterising them using X-ray crystallography and CryoEM. Our analysis shows striking differences in the organisation of even seemingly conserved components of kinetochore and evidences of Aurora-B kinase mediated regulation of kinetochore assembly.

130 – The glycosyltransferase Goe1 (Glucan Organizing Enzyme 1) contributes to cell wall integrity and echinocandin resistance

Sydney Briner

Sydney L. Briner and Tamara L. Doering

Washignton University in St. Louis

The lingering mystery of why Cryptococcus neoformans resists echinocandins, which inhibit Fks1-mediated synthesis of cell wall β-1,3-glucan, underscores how much of this organisms biology remains poorly understood. It also highlights the importance of identifying additional enzymes that contribute to cell wall integrity. We recently discovered the putative glycosyltransferase Glucan Organizing Enzyme 1 (Goe1). Cells lacking Goe1 (goe1) have defects in two cell wall glucans: α-1,4- and β-1,3-. Intriguingly, upon treatment with echinocandins, goe1 struggles to grow. To understand this, I am interrogating Goe1s enzymatic activity and how it influences Fks1. Surprisingly, I found that Goe1 resides in the Golgi. This suggests it does not work directly on cell wall glucans, although it could potentially impact Fks1 activity through several mechanisms. So far, I have found through RT-qPCR that FKS1 expression is not perturbed in goe1, while yeast two-hybrid studies suggest that Goe1 interacts with an intracellular domain of Fks1. This suggests that protein interactions may be involved, possibly relevant for Fks1 trafficking. Finally, I showed by imaging that Fks1 is mislocalized in goe1. One model to explain these disparate observations is that Goe1 acts in lipid glycosylation. Preliminary analysis of glycolipids extracted from wild-type and goe1 cells by thin layer chromatography and mass spectrometry suggests that Goe1 may contribute to the synthesis of glycoinositol phosphoceramide (GIPC) lipids, which are particularly elaborate in C. neoformans. Together, my data implicate Goe1 in GIPC synthesis and suggest a novel model for the role of cryptococcal GIPCs in Fks1 function and echinocandin resistance.

131 – Structural Basis for Functional Divergence of Actin in Cryptococcus neoformans

Freya Cardozo

Freya Cardozo (1), Kiran Vyas (2), Saikat Chowdhury (2), Saravanan Palani (1)

Indian Institute of Science, Bangalore

The actin cytoskeleton is a fundamental determinant of cell shape, polarity, intracellular trafficking, and morphogenesis. Although actin is among the most highly conserved eukaryotic proteins, accumulating evidence suggests that actins from different organisms possess specialized structural and biochemical properties that contribute to distinct cellular functions. Cryptococcus neoformans, a life-threatening opportunistic fungal pathogen, exhibits a distinctive actin cytoskeletal organization compared to model yeasts, yet the molecular basis underlying these differences remains unexplored. Here, we biochemically characterize Cryptococcus neoformans actin (CnACT) and resolve its filament structure to a 2.9 resolution using cryo-electron microscopy. Our analysis reveals differences in the conformation of the nucleotide-binding pocket, a substitution within the switch region, and multiple amino acid substitutions at otherwise conserved residues within the W-loopD-loop interface. These alterations are likely to influence filament dynamics and ABP interactions. Consistent with these observations, biochemical analyses revealed distinct polymerization kinetics compared to canonical actins. To establish a comprehensive framework for understanding cytoskeletal regulation in this pathogen, we have reconstituted the C. neoformans actin system in vitro through the purification of key ABPs, including tropomyosin, cofilin, profilin, formin, and the Arp2/3 complex. Ongoing studies using this reconstituted network will provide mechanistic insight into actin dynamics and their contribution to cellular organization and virulence in vivo. Together, these findings reveal previously unrecognized divergence within the actin family and identify structural features that may be exploited for the development of pathogen-selective antifungal therapeutics.

132 – Characterizing extracellular vesicle profiles in capsule mutants of the fungal pathogen Cryptococcus neoformans

Sofia Mutalova

Cassandre Deculty (1), Frédérique Moyrand (1), Pierre-Henri Commere (2), Guilhem Janbon (1) and Isabelle Mouyna (1)

RNA and Extracellular Vesicles Unit, Institut Pasteur, Inserm U1359 Mycologie Moléculaire et Médicale, Paris, FRANCE

Extracellular vesicles (EVs) are lipid bilayer-enclosed structures produced by cells that participate in the exchange of molecular information between cells. EVs act as carriers of proteins, nucleic acids, and lipids and are therefore involved in numerous physiological and pathological pathways. Although EVs have been widely studied in animals, plants, and even microorganisms, their characterization in fungi is limited. Most insights into fungal EVs originate from studies on Cryptococcus neoformans, an opportunistic pathogen that produces a polysaccharide capsule serving as its major virulence factor. In this study, we investigated mutants of genes involved in capsule biosynthesis for EV production. We identified both high and low EV producers, some also exhibiting differences in vesicle size and protein composition. Furthermore, we characterized their phenotypes under various drug treatments to explore potential associations with the observed differences in the EV producti on. Understanding the connection between EV biogenesis and the capsule formation may provide new insights into vesicle function and the pathways through which EVs are released into the environment. Key words: extracellular vesicles, capsule, Cryptococcus neoformans

133 – Mitotic and cytokinetic defects under stress can drive polyploidy in Cryptococcus neoformans

Claudia A Petrucco

Huarui Zhou1,3, Ashley Lim1,4, Steven B Haase1

1Department of Biology, Duke University, Durham NC, 2Tri-I MMPTP fellow

Polyploidy is observed in multiple human fungal pathogens and provides a strategy for rapid adaptation to stressful conditions encountered during infection. In Cryptococcus neoformans, polyploidy is a defining feature of Titan cells, which can reach ploidy levels from 2C to 312C. These cells exhibit enhanced survival compared to typical haploid yeast cells in the host. Polyploidy could arise through several mechanisms, but the precise cell-cycle alterations that underlie this process in C. neoformans remain poorly understood. Recently, work in the lab, demonstrated that cells exposed to hypoxia arrest before mitosis as large unbudded cells in G2. C. neoformans cells exposed to prolonged hypoxia either alone or combined with heat stress or nutrient limitation, exhibited mitotic and cytokinetic defects that drove polyploid cell formation. Live-cell imaging revealed several distinct mitotic and cytokinetic failures that subsequently produced polyploid cells with one or more nu clei. These observations reveal that host-relevant stresses can disrupt normal mitotic progression and promote polyploidization through multiple mechanisms. Using molecular approaches coupled with live-cell imaging, we aim to identify how core cell-cycle machinery is modified during polyploidization and how hypoxia contributes to these changes. Because the ability to grow and divide in host-imposed stress is a major virulence factor, understanding how polyploid cells form and proliferate will provide insight into how polyploid Titan cells contribute to disease progression and fungal pathogenicity.

134 – Regulation of Cryptococcus Argonaute by arginine methylation

Sueden Oliveira de Souza

1Kumar, K; 1Orrego, A; 1Van-Nues, R; 2Abreu-Goodger, C; 1Bayne, EH

University of Edinburgh

Cryptococcus fungi are opportunistic pathogens that can cause life-threatening infection in humans. An important factor in Cryptococcus virulence is its genetic variability, often derived from transposable element (TE)-mediated mutations. TE repression is commonly achieved via RNA interference (RNAi), a conserved gene regulatory pathway mediated by Argonaute (Ago) proteins and guided by small RNA (sRNA). In plants and mammals, some Argonaute family proteins can be regulated through arginine methylation catalysed by the protein arginine methyltransferase 5 (Prmt5), and subsequent binding by Tudor staphylococcal nuclease (TSN). Notably, Cryptococcus Agos carry the signature RGG-rich motif targeted by Prmt5 in other organisms, suggesting they may also be subject to methylation-dependent regulation. Consistent with this, our immunoprecipitation/mass-spectrometry analyses in Cryptococcus deneoformans identified that the TSN homolog coimmunoprecipitates with Agos, and found methy lation of the Agos RGG-rich motifs. Furthermore, deletion of the Ago1 RGG motif results in increased expression of the LINE1 retrotransposon, and decreased LINE1 sRNA accumulation . I further aim to dissect (i) the role of the RGG motif in Argonaute localisation and function and (ii) how methylation of the RGG motif contributes to its function. This study will shed light onto the post-translational regulation of Cryptococcus Argonautes, and their impact on TE repression.

135 – Perturbing Mps1 Kinase in the Cryptococcus neoformans titan cell, implications for a key morphological transition

Thomas Davies

Prof Kevin Hardwick* Prof A. Jeyaprakash Arulanandam**, Wulfrun Godson*

University of Edinburgh

C. neoformans ability to undergo a striking morphological transition within the human host to form large, polyploid titan cells presents a unique biomechanical challenge. How the polyploid titan genome is organised for the reductive divisions which occur from titans, and how this process is regulated via the mitotic or meiotic machinery is little understood. Additionally, titans are thought to be important for virulence in cryptococcal disease, representing a potential novel therapeutic target. Here, we investigate the requirement for components of the Spindle Assembly Checkpoint (SAC) - a key mitotic quality-control mechanism - in titan formation and division. The SAC monitors the attachment of chromosomes to spindle microtubules during meiosis and mitosis, preventing cell-cycle progression until all chromosomes are correctly aligned; errors in this process lead to aneuploidy and cell death. We hypothesise that the polyploid nature of titan cells may render them susceptible to perturbation of the mitotic machinery, with downstream consequences for viability and virulence. We find that the SAC is not required for titan formation or reductive divisions, but that a central SAC-associated kinase, Mps1, is essential for both. Perturbation of Mps1 leads to reduced titan formation, morphological and nuclear organisation defects, and severely decreased viability in the few titans that form. Furthermore, a chromosome 6 disomy is consistently observed in mps1 strains and is enriched among surviving mps1 titan daughters. These findings reveal a SAC-independent role for Mps1 in titan morphogenesis and ploidy control, and raise intriguing questions regarding the regulation of this unique morphological state.

136 – The Role of CD4+ T Cells in Hypervirulence of the Clinical Isolate UgCl422 of Cryptococcus neoformans

Bruno Borges

Paige Chenoweth, Kirsten Nielsen.

Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA

Cryptococcus neoformans is an environmentally acquired fungal pathogen and the leading cause of cryptococcal meningitis, a life-threatening disease that primarily affects immunocompromised individuals. Disease severity is shaped by both host genetic background and fungal virulence traits. Distinct clinical isolates can drive divergent disease outcomes depending on host immune context, including hypervirulent infections characterized by excessive inflammation. The clinical isolate UgCl422 induces hypervirulent disease in A/J mice, marked by elevated IFN-ɣ production and a mixed Type 1/Type 17 immune response, while causing typical disease in C57BL/6 mice. Although IFN-ɣ is generally protective during cryptococcosis, excessive levels correlate with worsened disease outcome, suggesting immune dysregulation as a driver of pathology. The cellular source of IFN-ɣ during UgCl422 infection remains unclear. Based on clinical observations that HIV-infected patients with depleted CD4+ T cells survived cryptococcal meningitis, we hypothesized that CD4+ T cells contribute to excessive IFN-ɣ production during UgCl422 infection. To test this, A/J mice were intranasally infected with UgCl422 or the reference strain KN99, followed by selective CD4+ T-cell depletion. Lung fungal burden, cytokine profiles, and survival were assessed, and CD4+ depletion was confirmed by flow cytometry. CD4+ T-cell depletion abolished the IFN-ɣ spike associated with UgCl422 infection and altered disease progression, demonstrating that CD4+ T cells are required for pathogenic IFN-ɣ responses. These findings reveal a critical role for CD4+ T cells in driving hypervirulence and provide insight into immune-mediated pathology during cryptococcal infection.

137 – To CT or Not CT?' That is the question, but LP is the answer

Enock Kagimu

Sara Wallace2, Biyue Dai3, Suzan Namombwe1, Timothy Mugabi1, Sarah Kimuda1, Jane Gakuru1, Tessa Adzemovic1,4,5, Morris K Rutakingirwa1, Samuel Okurut1, Fiona V Cresswell1,6,7, David R Boulware4, David B Meya1,4,8, Nathan C Bahr4

Infectious diseases Institute, Makerere University, College of Health Sciences, Kampala, Uganda

Background Lumbar puncture (LP) is required for diagnosis in those suspected of having meningitis, but carries risks. The utility of pre-LP brain imaging to identify space-occupying lesions and prevent adverse events is debated. Methods We prospectively enrolled Ugandan adults with HIV-associated meningitis from August 2013 to November 2024. All participants received a diagnostic LP upon hospital admission without prior head CT. Death within 24 hours post-LP served as a surrogate marker for assessing post-LP complications. Risk factors, including baseline laboratory results, focal neurological deficits, Glasgow Coma Scale (GCS) score, brain imaging findings, and meningitis etiology, were assessed. Results Of 3,552 participants, 83 (2.3%) died within 24 hours. GCS8 (16% (13/83) vs survivors, 4% (138/3469; p<0.001), higher median baseline CSF opening pressure (392 mmHO [IQR: 173542] vs survivors 200 mmHO [IQR: 125-300], p<0.001), and focal neurologic deficits 20% (16/80) vs survivors 10% (360/3,444; p=0.009) were associated with death within 24 hours post-LP. Focal neurologic deficits were associated with increased odds of 24-hour mortality (unadjusted OR=2.08, 95%CI 1.153.53; adjusted OR=3.10, 95%CI, 1.217.29). Of those with CNS imaging, 221 had a relative contraindication to LP on imaging, of whom 0.9% (2/221) died within 24 hours of LP versus 0.8% (2/259) mortality among those whose imaging showed no contraindication (p=>0.99) Conclusion In persons with HIV-associated meningitis, GCS<8 and focal deficits on clinical examination were associated with death within 24 hours; brain imaging findings contraindicating LP weren't. Few brain imaging studies were done in those who died within 24 hours.

138 – HIV-negative Colombian patients with Cryptococcus gattii and C. neoformans infection present autoantibodies neutralizing GM-CSF

Carolina Firacative

Carlos A. Arango-Franco (carlos.arango2@udea.edu.co) (1,2), Julian Rojas (julian.rojass@udea.edu.co) (1), Mélanie Migaud (melanie.migaud@inserm.fr) (3), Clara Inés Agudelo (cia1949@gmail.com) (4), José Luis Franco (jose.franco@udea.edu.co) (1), Jean-Laurent Casanova (casanova@rockefeller.edu) (3,5-9), Anne Puel (anne.puel@inserm.fr) (3,5,6), Jairo Lizarazo (jflizar@gmail.com) (9), Elizabeth Castañeda (ecastaneda21@gmail.com) (4), Andrés A. Arias (aaugusto.arias@udea.edu.co) (1,10).

School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia.

Background and aims: Cryptococcosis is a life-threatening disease caused by Cryptococcus neoformans or Cryptococcus gattii. While autoantibodies (auto-Abs) neutralizing granulocyte-macrophage colony-stimulating factor (GM-CSF) in otherwise healthy adults with cryptococcal meningitis have been described since 2013, these findings have been scarcely reported in Latin America. The aim of this study was to search for neutralizing auto-Abs in sera collected from Colombian patients with non-HIV-associated cryptococcosis, from a retrospective national cohort from 1997 to 2016. Methods: Clinical and laboratory records were reviewed from the National Surveillance on cryptococcosis, and the presence of neutralizing anti-GM-CSF auto-Abs in sera from 30 HIV-negative adults with cryptococcosis was assessed by flow cytometry. Out of the patients, 13 had infection caused by C. gattii and 17 by C. neoformans. Results: Auto-Abs neutralizing GM-CSF were detected in the sera of 10 out of 13 (77%) patients with C. gattii infection and one out of 17 (6%) patients infected with C. neoformans. Conclusions: This study emphasizes that, in HIV-negative patients, the presence of auto-Abs that neutralize GM-CSF is an important risk factor for the development of cryptococcosis, particularly caused by C. gattii, which, in turn, shows the differences in the epidemiology of cryptococcosis caused by the two species. Notably, the disease-causing factors of almost two-thirds of the studied patients remain unknown. Additional inborn errors of immunity may explain some of these cases.

139 – GM-CSF regulates monocyte and granulocyte response during Cryptococcus gattii infection

Alison Ricafrente

Sreemoyee Acharya*, Shuyi Chen*, Adiza Abass*, Aelita Arshakyan*, Tyler J. Olson*, Apurwa Trivedi*, Lena J. Heung*

Department of Medicine, Womens Guild Lung Institute, Cedars-Sinai Medical Center, Los Angeles, California, United States of America. ars-Sinai Medical Center

Autoantibodies against the pleiotropic cytokine GM-CSF have been linked to invasive infections by the fungal pathogen Cryptococcus gattii in apparently immunocompetent patients. Using a murine model of respiratory infection, this study investigated the potential role of GM-CSF (or CSF2) in the host immune response to C. gattii. Initially, we found that GM-CSF secretion is induced in the lungs of wild type (WT) C57BL/6J mice when given a fatal inoculum of C. gattii. In infected Csf2-/- mice, there is significantly decreased lung fungal burden, reduced inflammatory lung damage, and improved survival compared to WT. Conversely, intranasal administration of recombinant GM-CSF to WT mice increases lung fungal burden. We determined that GM-CSF promotes monocyte differentiation into alveolar and interstitial macrophages during C. gattii infection and that ablation of these cells in infected CCR2-DTR+ mice improves host outcomes, similar to Csf2-/- mice. Both alveolar and interstitial macrophages in WT mice skew towards M2 polarization, and in vitro, WT BMDMs exhibit poor anti-fungal activity against C. gattii. Additionally, we found that infected WT mice have increased eosinophil over neutrophil influx into the lung, in contrast to Csf2-/- and CCR2-DTR+ mice. Ablation of neutrophils in infected Mrp8cretg iDTR+ mic e increased lung fungal burden, indicating that the relative absence of neutrophils in infected WT lungs may also impair fungal control. Our results highlight that GM-CSF plays a detrimental role in host responses to C. gattii by regulating the presence of monocyte-derived macrophages and granulocytes in the lungs and subverting their effector functions to benefit the fungus.

140 – Cerebrospinal Fluid and Fungal Burden Associated Cellular Immune Responses in HIV-Related Cryptococcal Meningitis: From Sterile Fungal Cultures to Low and High Fungal Burden Disease

Samuel Okurut

Abduljewad Wele 5, James E Scriven 3, Tyler D. Bold 2, Annie Wilt 2, Mark Oilor 1, Morris K. Rutakingirwa 1,4, Caleb P Skipper 2, Laura Nsangi 1, Nathan C. Bahr 2, Suzan Namombwe 1, Jane Gakuru 1, Timothy Mugabi 1, Biyue Dai 5, Abdu K Musubire 1, David B. Meya 1,2,4*, David R. Boulware 2* (* -Equal contributions).

Research Department, Infectious Diseases Institute, Makerere University, Kampala, Uganda

Introduction Cryptococcal meningitis is a major cause of serious meningitis in people with advanced HIV. While the disease is linked to inflammation, little is known about how the fungal burden relates to the immune response in the central nervous system-associated neuroinflammation. Methods We studied 211 consenting adults presenting with HIV-related cryptococcal meningitis. We characterized baseline CSF cellular immune responses using spectral flow cytometry. We grouped participants into three unique visualized groups using unsupervised UMAP and PCA CSF quantitative fungal culture clusters: sterile (n=21), with low fungal burden (15,000 CFU/mL, n=77), and high fungal burden (>15,000 CFU/mL, n=113) groups separated at approximately the median CFUs. Results Higher CSF fungal burden was inversely associated with lower peripheral CD4+ counts, CSF white cell counts by microscopy, and CSF protein (all; P0.035). Subsequently, CSF fungal burden was inversely associated with CSF total CD3+ leukocytes, CD4+ T cells (r= -0.28), natural killer cells (r-0.13), neutrophils (r=-0.17), and dendritic cells (r=-16) as defined by flow cytometry (all Pearson r; P0.013). Fungal burden was positively associated with CD4+ T cells expressing Th1-polarized CXCR3+ chemokine receptor (median 24% high culture, 13% low, and 15% sterile groups; P=0.048), and immune checkpoint PD-1+ expression on CD8+ T cells (66% high culture, 61% low culture, and 53% sterile; P=0.009). Conclusion Higher CSF quantitative fungal culture burdens were associated with reduced absolute CD4+ T cells, NK cells, neutrophils, and dendritic cells, yet elevated PD-1 checkpoint expression among CD8+ T cells, and elevations in CD4+ T cells expressing Th1-polarized CXCR3+ inflammation-activated chemokine receptor.

141 – When Immunity Turns Pathogenic: Monocyte-Derived iNOS Protects Against Lethal Immunopathology in Cryptococcal Meningitis

Jintao Xu

Rylan Hissong, Maia Lintner, Alexander Ballesteros, Jane Barnett , Lauren Borhani, Shelby Hopson, Kristie Goughenour, Michal A Olszewski

Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Health System, Ann Arbor, Michigan, USA

Inducible nitric oxide synthase (iNOS) is a pivotal regulator of host defense, yet its role in balancing antimicrobial activity against tissue damage during Cryptococcal meningitis (CM) remains undefined. Here, we demonstrate that iNOS functions as an essential "brake" on lethal CNS inflammation rather than a primary antifungal effector. Using a murine model of CM, we found that Nos2-deficient mice infected with Cryptococcus neoformans (H99) exhibited fulminant neurological decline, rapid cachexia, and early mortality compared to wild-type controls. Crucially, this accelerated lethality was uncoupled from fungal burden, driven instead by extensive CNS immune infiltration and blood-brain barrier disruption. Using CCR2creNos2flox mice, we identified inflammatory monocytes as the obligatory source of protective iNOS. Mechanistically, the loss of nitric oxide-mediated feedback inhibition locked myeloid cells in a hyper-inflammatory state, driving uncontrolled CCL2, IFNɣ, and IL-1α/β production. This cytokine storm provoked pathological neutrophil recruitment. Strikingly, neutrophil depletion rescued Nos2-deficient mice from early mortality. These results mirror clinical observations in human CM cohorts, where reduced iNOS expression correlates with poor neurological outcomes despite antifungal therapy. Collectively, our data establishes iNOS as a vital suppressor of neurotoxicity, suggesting that host-directed immunometabolic therapies targeting the iNOS-NO axis may prevent lethal immunopathology in CM.

142 – In vivo assessment of host-pathogen interaction in the crossing the blood-brain barrier in murine model of experimental cerebral cryptococcosis correlating cellular with pathophysiological events

Lara Roosen

L. Musetta1, D. Maes1, S. Helsper1, M. Nobis2,3 , U. Himmelreich1

Biomedical MRI unit, Department Imaging and Pathology, KU Leuven, Belgium

Cryptococcus neoformans (CN) causes severe infections that can spread from the lungs to the central nervous system (CNS). The mechanisms by which CN crosses the bloodbrain barrier (paracytosis, transcytosis, or Trojan Horse mechanism) remain unclear. Clarifying early CNS entry and disease progression, a multimodal imaging approach combining Magnetic Resonance Imaging (MRI) and Two-Photon Intravital Microscopy (2P-IVM) was used to monitor CN dissemination longitudinally. Thirty Balb/C mice with an implanted cranial window were infected intravenously with 50.000 CN. After infection, mice were imaged for 8h, 24h, 48h using 2P-IVM. Lesions were confirmed by MRI on 4/7days. Influence on lesion formation was examined in depth (silicone oil vs. PBS, presence vs. absence of meninges, and impact of window placement). In mice infected with CNKN99E2-Crimson, cryptococci were observed after 1h with cluster after 5h with capillary bed occlusion. Mice infected with CNH99-EGFP showed similar results. Titan cells were confirmed at 24h and 48h with daughter cell production. After 7 days, lesions were observed in MRI in the cortex beneath the cranial window, allowing for imaging these lesions at cellular level with 2P-IVM, where indications of capillary vessel ruptures were observed. No differences were observed in lesion formation based on window placement (RW/LW) or compound used (PBS/silicone oil). Removing meninges didnt affect autofluorescence. We developed a multimodal 2P-IVM/MRI platform to track cerebral cryptococcosis across disease stages. Longitudinal cellular imaging revealed spread from onset to lesion formation, with indications of vessel rupture and transcytosis but no Trojan horse mechanism, highlighting the need for further studies.

143 – From the environment to the host, Cryptococcus neoformans and the building of a better model

Jennifer Tenor

Dena L. Toffaletti (1), Charles Giamberardino (1), Ayana Paul (2), Fred S. Dietrich (3), Joseph N Jarvis (4) , Christina A. Cuomo (5), John R. Perfect (1,3)

Duke University School of Medicine, Dept. of Medicine, Division of Infectious Diseases

Hypothesis: Cryptococcus neoformans clinical and environmental isolates can differ in their ability to cause disease, but the evolutionary factors underlying these differences remain poorly understood, in part due to the complexity of both the pathogen and the host. Methods: To address this knowledge gap, we evaluated infections caused by clinical and environmental isolates using an oropharyngeal cryptococcosis mouse model and multiple unique isolate collections to enable more detailed analyses of disease production. Results: Consistent with prior studies, we observed that consistently environmental isolates generally fail to cause severe disease comparable to that caused by aggressive clinical isolates. Unlike the environmental isolates, clinical isolates display a more varied virulence potential. Thus, we have assembled a library of 30 nonaggressive and 30 aggressive isolates comprised of both environmental and clinical strains of opposite mating types, different clades, and sources. This collection supports whole-genome sequence analysis (WGS), genome-wide association studies (GWAS), and phenotypic characterizations to identify factors that influence disease outcomes in a robust mouse model. In addition, using a new mouse model for the interrogation of host model responses, we compared granuloma formation between nonaggressive and aggressive yeast isolates and found that aggressive isolates induced robust host granuloma formation within 21 days of infection, whereas nonaggressive isolates did not. Conclusion: This work highlights the importance of the disease potential of individual yeast isolates for understanding the evolution of virulence through genetic, genomic, and host-pathogen studies and provides additional evidence to the observation that environmental isolates tend to be less aggressive.

144 – Long-term evaluation of cryptococcal dissemination from pulmonary infection to late-stage neurological complications in an optimized rat model with orotracheal inoculation

Dries Maes

S. Helsper (1); Lara Roosen (1); M. Meyer (2); K. Lagrou (3); U. Himmelreich1

KU Leuven

Currently, mouse models that use intravenous inoculation is the gold standard for studying cryptococcal dissemination due of its high throughput and reproducible workflow. To more closely mimic the natural route of infection, we aimed to optimize the implementation of a rat model after intratracheal (IT) infection. Rather than an artificial bloodborne dissemination, this IT model starts from a primary pulmonary infection, as described in clinical cases. Furthermore, this rat model provides many advantages related to immunological aspects and follow up by in vivo imaging. Female Fisher 334 rats were orotracheally infected (1.000.000 cells/200 L; n = 6) with either C. gattii (R265) or C. neoformans (KN99a) as previously described by Meyer et al. Pulmonary infection was followed-up weekly by micro-CT (SkyScan 1278). To assess cerebral lesion formation and development of meningitis, anatomical (T2w) and diffusion weighted MR imaging (ADC) was used (9.4T MRI scanner, Biospec 94/20, Bruker). Weekly CT acquisitions revealed a clear increase in pulmonary fungal burden from week 5 p.i. onwards, and evidence of neurological involvement in the form of cerebral lesions was found on MRI acquisitions from week 7 p.i. These results indicate that the OT rat model is ideal to monitor cryptococcal dissemination and disease progression. Rats kept stable until week 10 p.i. despite incremental increase in fungal dissemination, proving that infection can be sustained without immunosuppression, contrary to previous literature reports. This model may allow us to monitor disease development and dissemination longitudinally without the restrictions of the current standard mouse model.

145 – Unbiased protein correlation profile analyses unravel putative virulence-associated protein complexes in Cryptococcus neoformans

Mayara Barbara da Silva

Florence Roux-Dalvai (2), Arnoud Droit (2), Jennifer Geddes-McAlister (1)

Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

Protein-protein interactions (PPIs) are crucial for understanding critical biological processes in pathogenic species, such as Cryptococcus neoformans. Current methods used to characterize such interactions either restrict analysis to a single protein of interest or may overestimate interactions due to complex global proteomics data. Therefore, co-fractionation methods offer an alternative for globally assessing PPIs in a simplified environment, providing a comprehensive understanding of the mechanisms driving cryptococcosis. As a result, I am designing a novel platform for investigating PPIs in C. neoformans that can help identify crucial players in virulence. To achieve this goal, I have developed a co-fractionation method based on size exclusion chromatography coupled with mass spectrometry to enhance the detection of true interactors in secreted and cellular protein samples (i.e., secretome and proteome, respectively). PPI predictions were then inferred via the STRING n etwork database to highlight known and novel physical protein complexes within functional clusters. As a result, 216 and 1699 unique proteins were identified, spread across 24 secretome and proteome fractions, respectively. Notably, predicted secretome networks include vesicle-associated proteins that bind stress-response proteins, indicating a role in extracellular defense. Furthermore, CNAG_00287 was inferred to play a role in thermal stress, as it was found to bind heat-shock proteins (CNAG_06208 and CNAG_03944) in both the secretome and proteome networks. Further in silico and in vitro validations will be carried out to characterize this novel putative heat-shock protein fully. Overall, this project delivers a new method capable of comprehensively detecting interacting proteins essential for C. neoformans pathogenicity.

146 – Using human airway and alveolar organoid-derived monolayers to quantify parameters of early cryptococcal infections

Sebastian Reusch

Emily Korsch, Jumana Ibrahim-Bacha, Charlotte Schwister, Anton Aebischer, Christian Klotz, Volker Rickerts

Robert Koch-Institute, Department of Infectious Diseases, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany

Background and aims Cryptococci are environmental fungi that cause localized or disseminated infections typically after uptake via the respiratory tract. Studies on pathogenicity are based on animal models, cancer cell lines or immune cells. However, to understand pathomechanisms novel models are required. Methods We cocultured cryptococcal reference strains (C. gattii VGII/R265 and C. neoformans VNI/H99) with organoid-derived monolayers (ODMs) originating from adult stem cells of proximal (= Airway) and distal (= Alveoli) human respiratory tissue. Barrier function of ODMs was probed by measuring the transepithelial electrical resistance. Internalization of fungi into respiratory cells was visualized by immunofluorescence stainings. Fungal viability was assessed by cell counting and cultivation. Ink stainings were used to quantify fungal cell morphology. Results While barrier function of airway ODMs was maintained, cryptococci induced a barrier breakdown in alveolar ODMs. Immunofluorescence stainings revealed that cryptococcal adhesion and internalization events to and into in airway ODMs were rare, while alveolar ODMs showed an increased susceptibility towards cryptococcal internalization. Both cryptococcal strains were able to proliferate on ODMs. Analysis of fungal morphology revealed strain specific fungal adaptation with giant cells in C. gattii and pseudohyphal growth for C. neoformans while cocultured with alveolar, but not with airway ODMs. Conclusions Studying interactions of cryptococci with primary human cells of the respiratory tissue is feasible with Cryptococcus suggesting different fungal-host interactions of C. gattii and C. neoformans and specific tissue reaction in proximal versus distal tissues. Future studies will be used to characterize immunologic response and analysis of fungal subpopulations.

147 – Investigating the Role of Phosphatidylinositol 3-phosphate in the Cryptococcal Containing Phagosome

Kassandra Arias-Parbul

Peter Stuckey, Felipe Santiago-Tirado

University of Notre Dame, Department of Biological Sciences, Notre Dame, Indiana, United States of America

Cryptococcus neoformans ability to survive within the phagosomes of immune cells is one of the main drivers of infection. These cryptococcal-containing phagosomes (CCPs) within macrophages allow for a permissible environment for the fungi. Although studies have begun to characterize the fungal factors contributing to the CCP, there is a major knowledge gap in the specific host factors that influence its formation. We have found that a significant population of CCPs are labeled with both early and late endosomal markers. One of these, the host lipid phosphatidylinositol 3- phosphate (PI3P), is required for the proper maturation of phagosomes. However, reports from other microbial pathogens suggest that prolonging its association to the pathogen-containing phagosome inhibits its maturation. The purpose of this study is to determine the effect of the presence of PI3P on the CCPs properties. THP-1 cells are infected with KN99 C. neoformans and BY4741 S. cerevisiae for up to 8 hours. Cells are then stained with antibodies against EEA1, an early endosomal marker, or LAMP1, a late lysosomal marker. Up to 30% of all CCPs exhibit a hybrid state (PI3P and LAMP1) solely in cells infected with KN99 compared to BY4741 infected cells. While the presence of PI3P is unsurprising, its extended presence could be causing a delay in the fusion of the maturing phagosome with lysosomes which would be required to acidify. This, in turn, could promote the establishment of a permissive niche. We plan to further investigate this phenomenon using chemical inhibition or knock-down of the PI3P kinase.

148 – Innate Immune Responses Differ in Latent, Hypervirulent, and Typical Clinical Isolates of Cryptococcus neoformans

Analis Smith

Dr. Bruno Borges, Dr. Kirsten Nielsen

Virginia Polytechnic Institute and State University

Cryptococcus neoformans is an environmental fungal pathogen that primarily causes disease in immunocompromised people, including HIV/AIDS patients and those undergoing organ transplants or chemotherapy. It is known that infections caused by different clinical isolates of C. neoformans can lead to different disease manifestations; we classify these manifestations based on disease presentation severity in the inhalational mouse model of cryptococcosis as either typical, hypervirulent, or latent. Immunological responses between these three categories vary typical strains induce a detrimental type 2/Th2 mediated-response, latent strains have a beneficial type 1/Th1 mediated-response, and hypervirulent strains cause a detrimental overreactive type 1/type 17 response. It is unknown whether these differences in immune polarization are occurring as a result of early innate or adaptive immunity. To test the hypothesis that differential immune responses correlate with early innate responses, we conducted in vitro phagocytosis assays using bone marrow-derived macrophages and dendritic cells isolated from A/J mice and typical, latent, and hypervirulent C. neoformans isolates. Our results show a lower phagocytosis rate in the hypervirulent UgCl 422 and a higher phagocytosis index in the latent UgCl 223 compared to the typical reference strain KN99. These data suggest that early innate phagocytosis rates correlate with immune polarization and subsequent disease outcomes. This early innate response may influence antigen presentation and subsequent immune polarization during the C. neoformans infection.

149 – Dynamic Interactions Between Cryptococcus spp. and Human Lung Organoids During Infection.

SUELEN ANDREIA ROSSI

Sulen Andreia Rossi1; Gabrieli M. Huff1; Evelin Brandao da Silva2; Kayo Schemiko Almeida1; Joo Silvrio Larguesa1; Tatiana P. Coutinho1; Gabriel P. L. da Silva1; Patrcia Shigunov2; Flavia C G Reis1; Ricardo Amorim1; Carlos P. Taborda3; Marcio Loureno Rodrigues1; Lysangela Ronalte Alves1.

Carlos Chagas Institute, FIOCRUZ, PR, Brazil.

In recent years, there has been an increased use of human organoids in several studies. However, the analysis of fungal infections using organoid models are still scarce. Therefore, the aim of this work is to evaluate the interaction between Cryptococcus spp. and human lung organoid, as well as to assess the effects of extracellular vesicles produced by organoid cells during the infection process. The lung organoids were obtained from human embryonic stem cells (hESCs) using cell differentiation protocols previously established in our group. Infection was performed using the H99 strain of Cryptococcus neoformans and the R265 strain of Cryptococcus deuterogattii. As preliminary results, analysis of post-infection images with the R265 strain demonstrated that fungal invasion occurred after 1.5 hours of contact, similarly to what was observed for the H99 strain. In addition, greater fungal recovery of both H99 and R265 strains was observed after 24 hours of infection. The size of fungal cells recovered after infection was also evaluated. For both strains, an increase in total cell size, cell body size and capsule size was observed. The H99 strain showed increased mainly after 1.5 hours of infection, whereas the R265 strain exhibited an increase in total cell size beginning in 6 hours after infection, reaching approximately 20 m, with some cells exceeding this size. These results contribute to a better understanding of the pathogenesis of cryptococcosis and may help elucidate the role of extracellular vesicles originating from lung tissue during fungal infection.

150 – Rab20 conveys immune signaling to the cryptococcal-containing phagosome during Cryptococcus infection of macrophages.

Felipe H. Santiago-Tirado

Peter V. Stuckey and Georgina Agyei

University of Notre Dame

Cryptococcus neoformans causes ~200,000 deaths yearly, mostly in immunocompromised individuals. It is acquired through inhalation and lung macrophages are the main line of defense. The interactions with these cells will determine the outcome of the infection, influencing if the lung macrophages will serve as safe haven for replication and promote extrapulmonary dissemination, both of which are associated with mortality. The mechanisms C. neoformans use to mediate this intracellular parasitism and how it is influenced by the immune response are not fully understood. Therefore, understanding these interactions and elucidating the properties of the cryptococcal-containing phagosome (CCP) are critical steps needed to improve the management of this disease. We have identified three distinct populations of CCPs based on acidification patterns: those that remain acidic, those that lose acidity, and those that never acidify. Unlike phagosomes containing S. cerevisiae, CCPs exhibit significant phagosomal membrane damage and maturation delay. Moreover, a subpopulation of CCPs display both early endosome (PI3P) and lysosome (LAMP1) markers, suggesting that C. neoformans can alter its phagosome and provides a potential mechanism for intracellular survival that may be driving cryptococcal pathogenesis. Notably, when macrophages are stimulated with interferon-ɣ, acquisition of lysosomal markers occurs more rapidly and more CCPs acidify. Host Rab20 has been shown to be interferon-ɣ -inducible and appears to be critical for this promotion of phago-lysosomal fusion as knockdown of Rab20 delays maturation of phagosomes containing non-pathogenic cargo. Therefore, Rab20 may be linking host immune status and CCP properties during infection, playing a critical role in cryptococcal infection outcome.

151 – Phenotypic landscape of Cryptococcus neoformans drives mechanistic interrogation of virulence factors

Michael Boucher

Sanjita Banerjee (1), Meenakshi B. Joshi (1), Angela L. Wei (1), Matthew J. Nalley (1), Manning Y. Huang (1), Susan Lei (1), Massimiliano Ciranni (2), Andrew Condon (3), Andreas Langen (3), Thomas D. Goddard (3), Katherine J. Susa (3), Vito Paolo Pastore (2), Balyn W. Zaro (3), Hiten D. Madhani (1)

Division of Infectious Diseases, Wadsworth Center, New York State Department of Health

Cryptococcus neoformans is a pathogenic basidiomycete yeast that diverged from well-studied fungi in the phylum Ascomycota at least 450 million years ago. Defining functions for basidiomycete-specific genes is essential for deciphering mechanisms of cryptococcal pathogenesis, yet doing so at the genomic scale remains challenging. We performed massively parallel fitness screens of 4328 single-gene C. neoformans deletion strains in 141 unique in vitro growth environments and in a mouse infection model. In vivo screens revealed 574 fungal genes required for fitness during murine pulmonary infection. Hierarchical clustering of mutant fitness profiles produced a phenotypic atlas of this organism, reconstructing core molecular pathways and assigning validated cellular roles to unstudied genes. Integrative analysis of these datasets revealed a novel virulence gene, RGH1 (Required for Growth in Host-like conditions), uniquely required for fitness during tissue culture and in vivo i nfection. Concordantly, RGH1 transcription is massively induced upon shifting to host-like growth conditions. We pursued the function of this factor though a genome-scale CRISPR/Cas9 suppressor screen and affinity purification-mass spectrometry experiments, uncovering a putative role in cell wall alpha-glucan biogenesis. Together, our results suggest a model in which Rgh1 is actively deployed upon host entry to drive adaptive cell wall remodeling that is critical for tolerating infection-relevant stressors. This work highlights the power of high-throughput genetics in linking unbiased discovery with mechanistic studies, providing an experimental framework for interrogating uncharacterized fungal virulence determinants.

152 – Longitudinal in vivo bioluminescence imaging reveals a protective role for MARCO deficiency in cryptococcal clearance and fluconazole efficacy

Greetje Vande Velde

Eliane Vanhoffelen (1), Lander De Herdt (1), Lauren Michiels (1), Agustin Reséndiz-Sharpe (1), Chinaemerem U Onyishi (2,3), Laura Seldeslachts (1), Tine Van Win (4), Kasia Błażejczyk (1), Lara Roosen (1), Flore Belmans (1), Katrien Lagrou (4,5), Subhankar Mukhopadhyay (6), Siamon Gordon (7,8), Robin C. May (2), Greetje Vande Velde (1)

KU Leuven

Background: Cryptococcus neoformans is an opportunistic fungal pathogen that can disseminate from the lungs to the CNS, causing life-threatening meningoencephalitis. The role of cryptococcal vomocytosis from pulmonary macrophages in dissemination remains unclear. Macrophage Receptor with Collagenous structure (MARCO) was recently identified as a potent negative regulator of cryptococcal vomocytosis in vitro, with its absence strongly enhancing expulsion rates. Here, we investigated the in vivo role of MARCO in cryptococcal infection and fluconazole responsiveness.

Methods: Wild-type and MARCO-KO C57BL/6 mice were orotracheally infected with a low (50 cells) or higher (5000 cells) inoculum of luciferase-producing KN99 C. neoformans. Fluconazole 80 mg/kg/day was started 1.5 days post-infection and switched to 40 mg/kg/day after 8 days. To non-invasively track real-time fungal burden over 4.5 weeks, we developed a novel bioluminescence imaging (BLI) method using 500 mg/kg D- luciferin. Results: MARCO deficiency enhanced fluconazoles antifungal efficacy, significantly reducing brain dissemination and fungal burden compared to wild type controls. MARCO-KO mice also cleared low inoculum pulmonary infection more effectively, delaying or preventing CNS spread, but showed no protection at higher fungal inocula unless combined with fluconazole. These effects are potentially driven by increased extracellular fungal exposure via enhanced vomocytosis in MARCO-KO mice, improving antifungal access.

Conclusion: Our findings identify MARCO as a host factor influencing early cryptococcal clearance and antifungal potentiation and highlight longitudinal BLI as a powerful tool for studying infection and treatment dynamics in vivo. Combining host-directed MARCO targeting with antifungal therapy may offer a novel strategy to prevent cryptococcal dissemination, warranting further investigation.

153 – Identification of drug targets for Cryptococcus neoformans drug discovery

Master Chisale

1. University of Dundee, School of Life Sciences, Drug Discovery Unit, Biological Chemistry and Drug Discovery, Dundee, Scotland, United Kingdom, 2. Mzuzu University, Faculty of Science, Technology and Innovations, Biological Science Department, Mzuzu, Malawi

Cryptococcus neoformans (Cn) is a pathogen with global distribution and is a primary cause of mortality and morbidity among immunocompromised individuals. Limited accessibility, toxicity, and resistance to available antifungal classes are key challenges. Consequently, there is an urgent need to develop novel therapeutic options targeting previously unexploited pathways. This study was therefore conducted to identify a potential drug target against Cn and related pathogenic fungi. Published essentiality data were used as a starting point for target identification. FungiDB and NCBI databases were used to identify fungal orthologs and human homologs, respectively. The identified targets were further characterised using multiple databases, including BRENDA, UniProt, PDB, AlphaFold, and FungiDB, to evaluate which targets have published 3D structures, are likely to be expressed and purified, are tractable, and are enzymes. fPocket, PyMOL, ChimeraX, JALVIEW and UniProt were used to assess the druggability of each target. All targets with druggable substrate-binding pockets were evaluated for assayability using InterPro, SciFinder, UniProt and literature. Cn has 1,465 essential targets, of which 113 lack human homologs and are also essential in Candida albicans. Seventy-nine of these targets are deemed tractable, of which 40 are enzymes, and nine exhibit druggable pockets with well-aligned binding and active sites. Five targets are identified as readily assayable, with Thioredoxin reductase (TrxR) demonstrating the most favourable attributes. The majority of the potential drug targets identified in this project are novel and have promising profiles, worthy of further exploration for antifungal drug discovery.

154 – Uncovering Genetic Drivers of Biofilm Formation in Cryptococcus neoformans to Enable Antibiofilm Drug Discovery

Oscar Romero

Davier Gutierrez-Gongora (1), Norris Chan (1), Lauren Segeren (1), Eric Soubeyrand (1), Tarik Akhtar (1), Susan S. Bird (2), Brandon Bills (2), Rahul Deshpande (2), Stephanie N. Samra (2), Jennifer Geddes-McAlister (1)

Department of Molecular and Cellular Biology, University of Guelph, ON, Canada

Fungal pathogens form biofilmsstructured communities of cells embedded in a self-produced extracellular polymeric matrixthat enhance survival under environmental stress and contribute to persistent infections. In the human fungal pathogen, Cryptococcus neoformans, biofilm formation is associated with reduced antifungal susceptibility, often requiring higher drug doses or surgical intervention. For cryptococcal biofilms, key structural components shared with the polysaccharide capsule (a major virulence factor of C. neoformans) influence biofilm formation. While capsule-deficient strains frequently exhibit impaired biofilm formation, the molecular determinants driving this process remain incompletely understood. This study aims to identify proteins involved in cryptococcal biofilm formation and discover novel antibiofilm molecules targeting them. Using a forward genetics approach, we screened a C. neoformans var. grubii KN99 deletion library of 4,030 mutants (Madhani's Library) with a high-throughput biofilm quantification assay. Nineteen deletion strains exhibiting greater than 50% reduction in biofilm formation were prioritized for further analysis. These mutants were evaluated using additional phenotypic assays, including capsule production, thermotolerance, stress response, and virulence in the Galleria mellonella infection model. In parallel, proteins encoded by prioritized genes are being evaluated as therapeutic targets using a target-based drug discovery strategy. AlphaFold-predicted protein structures are employed for virtual high-throughput screening (vHTS) to identify small molecules with high predicted affinity for biofilm-associated targets. Computational screening is complemented by state-of-the-art mass spectrometrybased identification and characterization of bioactive compounds. Collectively, this work uncovers previously unrecognized molecular determinants of cryptococcal biofilm formation while discovering novel antibiofilm molecules using integrated computational and experimental drug discovery strategies.

155 – Repurposing Benzimidazole to Inhibit a Dissemination-Prone Morphotype of Cryptococcus neoformans

Eric Cheung

Dr. Jessica Brown

The University of Utah

Mortality from Cryptococcus neoformans primarily results from fungal dissemination from the lungs to the brain. We previously demonstrated that a small morphotype (10 m total diameter) termed seed cells, forms in the lungs and appears in the brain early in the dissemination process. These cells exhibit an enhanced capacity for dissemination and the establishment of new infections in extrapulmonary tissues. Here, we report 24 small-molecule hits from a drug repurposing screen that significantly inhibited seed cell formation and propagation (p 0.005). The strongest inhibitors albendazole, fenbendazole, and mebendazole are benzimidazole anthelmintics. In C. neoformans, this appears to disrupt normal cell development, resulting in enlarged cells and defective division. Benzimidazole treatment reduced seed cell formation from ~40% to ~5% and decreased established populations to ~4% within 24 hours. These findings suggest that benzimidazoles may inhibit the dissemination of C. neoformans and represent a promising class of compounds for treatment. While seed cells are confirmed, mechanisms of their formation and self-propagation capacity remain unclear. We hypothesize seed cells are the daughters of a normal mother cell under seed-cell-inducing conditions. To address this, live-cell imaging will visualize seed cell production and their ability to produce identical morphotypes. During dissemination from the lung, seed cells encounter various tissues, blood, and cerebrospinal fluid. Using media mimicking these conditions, we will measure seed cell production to determine how these microenvironments influence the preservation of this morphotype. Elucidating mechanisms of seed cell formation and propagation provides a novel strategy to halt progression of cryptococcal meningitis.

156 – Repurposing Acetylsalicylic Acid for Photodynamic Treatment of Cryptococcal Infections

Adepemi O. Ogundeji

Nozethu Mjokane, Olufemi S Folorunso, Carolina H Pohl, Martin M Nyaga and Olihile M Sebolai

University of the Free State

Photodynamic treatment (PDT) is often successful when used against aerobic microbes, given their natural susceptibility to oxidative damage. To this end, the current study aimed to explore the photodynamic action of acetylsalicylic acid (ASA; aspirin, which is commonly used to treat non-infectious ailments), when administered to respiring cryptococcal cells. The treatment of cryptococcal cells, i.e., exposure to 0.5 or 1 mM of ASA in the presence of ultraviolet light (UVL) for 10 min, resulted in a significant ($p < 0.05$) reduction in the growth of tested cells when compared to non-treated (non-Rx) cells, i.e., no ASA and no UVL. The treated cells were also characterised by diseased mitochondria, which is crucial for the survival of respiring cells, as observed by a significant ($p < 0.05$) loss of mitochondrial membrane potential ($\Delta\Psi M$) and significant ($p < 0.05$) accumulation of reactive oxygen species (ROS) when compared to non-Rx cells. Moreover, the photolytic products of acetylsalicylic acid altered the ultrastructural appearance of treated cells as well as limited the expression levels of the capsular-associated gene, CAP64, when compared to non-Rx cells. The results of the study highlight the potential use of ASA as a photosensitiser that is effective for controlling the growth of cryptococcal cells. Potentially, this treatment can also be used as an adjuvant, to complement and support the usage of current anti-microbial agents.

157 – Tackling the Many Faces of Cryptococcus: Hunting for Novel Antifungals

Irene Molina Privado

Irene Garca 1, Emiliana dOria 1, Juan Salamanca 1, Ana Lisa Valenciano 1, Delia Blanco-Ruano 1, Mara Marco 1, Tim Miles 1, Mara Pilar Manzano 1, Mischka Mooddley 1, Raafiah Haroon 1, Stephanie Gresham 1, Keely Anderson 1, Juliana Da Silva 2, Marta Garca 2, Beatriz Rodrguez 1, Manu De Rycker 2

GSK (Global Health Medicines R&D, GSK, Tres Cantos, 28760 Madrid, Spain)

Cryptococcus neoformans is listed as critical on the WHO 2022 Fungal Priority Pathogens list. This yeast causes cryptococcal meningitis, which is associated with high mortality among immunocompromised patients (accounting for 19% of HIV-related deaths) and has a high incidence in low- and middle-income countries. During infection, C.neoformans generates different morphotypes of varying sizes, possesses a polysaccharide capsule as its main virulence factor and can survive intracellularly. These characteristics produce variable drug susceptibilities that reduce compound activity, often aggravated with infection diagnosis at late stage. Limitations of available antifungal therapy include low availability and affordability, poor efficacy, toxicity and resistance. Despite the unmet clinical need, drug discovery activity is limited. A new Wellcome-funded project bringing together GSK, the University of Dundee and the University of Exeter is aiming to deliver new pre-clinical cand idates for cryptococcal meningitis. To identify starting points with a high probability of success, an in vitro assay that mimics physiologically-relevant conditions was developed. In this assay the yeast cells present as a heterogenous population aking to what is seen in animals and patients, with encapsulated cells of various sizes. Here we present the deployment of this assay to carry out a high throughput screening campaign at GSK. Approximately 950,000 compounds were tested in this assay, delivering hundreds of compounds with both activity against the yeast and selectivity over human cells. Although some compounds were related to azoles, around a hundred resulted in new chemical diversity, distributed across multiple clusters and numerous singletons.

158 – Wasp venom-derived peptides exhibit antifungal activity against Cryptococcus neoformans

Larissa Fernandes

Karina S. Simon1, Luisa C. Coelho1, Raffael J. A Castro1,2, Rafaela L. Souza4, Gabrielly B. Trindade4, Larissa Fernandes1,5, Mércia R. Mortari6, Anamélia L. Bocca1

Bi-Institutional Translational Medicine, Oswaldo Cruz Foundation, São Paulo, Brazil and Center of Biotechnology, University of Brasília, Brasília, Brazil.

Antimicrobial peptides (AMPs) constitute a diverse class of multifunctional bioactive molecules that exhibit broad-spectrum antimicrobial, antineoplastic, and immunomodulatory activities and have emerged as attractive candidates for the development of next-generation antifungal agents to overcome antifungal resistance, host toxicity, and the limited efficacy of currently available therapies. In this study, we evaluated the antifungal activity of the mastoparan peptides Polybia-MPII, isolated from the venom of Pseudopolybia vespiceps testacea, and Agelaia-MPI, derived from Parachartergus fraternus venom, against the human pathogenic yeast Cryptococcus neoformans. Both Polybia-MPII and Agelaia-MPI displayed potent in vitro fungicidal activity, which was associated with increased fungal plasma membrane permeability and significant alterations in cellular biophysical properties, including cell size and surface roughness. Combination assays revealed additive interactions between the two peptides and between each peptide and fluconazole. Notably, disruption of established C. neoformans biofilms was observed only at high peptide concentrations. In the Galleria mellonella infection model, treatment with either peptide significantly increased larval survival, exceeding 80% in comparison with the untreated control group. Collectively, these results demonstrate that Agelaia-MPI and Polybia-MPII exhibit relevant antifungal and in vivo protective activities, supporting their further development as lead compounds for novel antifungal strategies and highlighting wasp venomderived mastoparan peptides as promising components of therapeutic formulations for the treatment of cryptococcosis.

159 – Use of Oteseconazole for Consolidation Therapy and Secondary Prophylaxis in Fluconazole Non-Susceptible Cryptococcal Meningitis.

Sarah Kimuda

Joseph Owori1, Faizo Ssekindi1, Kisakye Diana Kabbale1, Cynthia Ahimbisibwe1, Jane Frances Ndyetukira1, Rhona Muyise1, Grace Menya1, Andrew Akampurira1, Tonny Luggya1, Mable Kabahubya1, Steve Brand 4 , David R Boulware2, David B Meya1,2,3, Mahsa Abassi2

Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda

Background: Fluconazole non-susceptible Cryptococcus neoformans (MIC 16 g/mL) represents a significant therapeutic challenge in culture-positive relapse due to the absence of alternative oral consolidation therapies. Oteseconazole, a tetrazole CYP51 inhibitor with potent in vitro activity against Cryptococcus (MIC 0.125 g/mL) has a prolonged half-life of 138 days. Oteseconazole offers a promising option for fluconazole non-susceptible cryptococcosis. Methods: In Kampala, Uganda, we enrolled a compassionate-care cohort for individuals with culture-confirmed cryptococcal-relapse due to fluconazole non-susceptible isolates (MIC 16 g/mL). After re-induction with liposomal amphotericin-based therapy, participants received oteseconazole 600 mg twice daily for 10 days as loading dose and then weekly 600 mg for 4 weeks. We assessed clinical course, CSF sterility, and recurrence. Results: Two individuals with culture-positive cryptococcal-relapse due to fluconazole non-susceptible Cryptococcus initiated oteseconazole therapy. Case 1 presented on antiretroviral therapy with CD4 count of 294 cells/L and a third episode of cryptococcal-relapse. CSF culture grew 6,500 CFU/mL at relapse with a fluconazole MIC=256 g/mL. Case 2 presented on antiretroviral therapy with CD4 count of 124 cells/L. CSF fungal culture grew 195,000 CFU/mL at relapse with a fluconazole MIC=32 g/mL. Both participants achieved clinical stabilization and CSF sterility. To date, >2 months of follow-up, no further episodes of meningitis, disease recurrence, or drug-related toxicity have been observed during follow-up. Conclusions: Early experience suggests that oteseconazole may provide effective consolidation therapy and secondary prophylaxis in cryptococcal meningitis. These preliminary observations support further systematic evaluation of oteseconazole as a novel oral agent for cryptococcosis. Enrollment is ongoing.

160 – Rsp5-mediated ubiquitination of a functional analog of the Rim8 arrestin facilitates Rim pathway activation in Cryptococcus neoformans

Marnus du Plooy

Lukas M. du Plooy1,2, Corinna Probst1, Matthew E. Capobianco1, Charles Giamberardino1, Claudia A. Petrucco2,3, J. Andrew Alspaugh1,2,3

1Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA
2Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, North Carolina, USA
3Department of Cell Biology, Duke University School of Medicine, Durham, North Carolina, USA

Pathogenic microorganisms use varied cellular processes to adapt to the stresses encountered in the infected host. These stresses include rapid alterations in ambient temperature, nutrient availability, and extracellular pH. Fungal pathogens, therefore, rely on the activation of stress response pathways such as the Pal/Rim pathway to adapt to the neutral pH encountered when infecting mammals. While this pathway is conserved among human pathogenic fungi, the proteins required for pH sensing appear to have diverged between different fungal phyla. Cryptococcus neoformans employs a pH-sensing protein in its Rim pathway that is distinct but functionally analogous to related proteins in well-studied ascomycete fungal systems. We determined that protein ubiquitination mediated through the Rsp5 ubiquitin ligase is required for C. neoformans virulence and for microbial adaptations to host-relevant conditions, including growth at host pH levels. C. neoformans Rsp5 is specifically required for Rim pathway activation. Using an unbiased screen for proteins that are ubiquitinated by Rsp5 in acidic and alkaline pH, we identified a new component of the C. neoformans Rim pathway that is targeted by Rsp5 for ubiquitination and shares protein features with the ascomycete Rim8/PalF proteins. Rsp5-mediated ubiquitination facilitates protein interactions with Vps23, a downstream trafficking component of the Pal/Rim pathway. Therefore, we define adaptation to ambient pH as one component of the broad cellular roles of Rsp5-mediated ubiquitination, as we explore how protein ubiquitination affects cryptococcal cell physiology, virulence, and microbial interactions with the host.

161 – Cryptococcal antigenaemia screening and treatment: observations from the field in South Africa (CAST OFF-SA)

Rhys Wenlock

Asha Thombrayil1, Raphaela Berghammer-Böhmer1, Marise Bremer1, Danielle Verhagen1, Richard Halley-Stott1,2, Tshiamo Mmotsa1, Shirley Lebenya1, Isabella Fatti1, Nkosingiphile Bongiwe Nxumalo3, Pauline Mbutuma1, Rudzani Mashau1, David Kabanda1, Juliet Kortrooi1, Lethukuthula Zondi1, Pamela Sithole1, Lia Edkins1, Phume Ngcobo1, Ntwenhle Makhaza1, Jeremy Nel4,5, John Black6, Thomas S Harrison7, Joseph N Jarvis8,9, David S Lawrence8,9, Nelesh P Govender1,7,8,11, Síle F Molloy1,7, Kyla Murphy1,2, Rhys D Wenlock12.

Wits Mycology Division, University of the Witwatersrand

Background and aims Early detection and treatment of cryptococcal antigenaemia (CA) among patients with advanced HIV (defined as CD4 count<200 cells/µl) prevents deaths due to cryptococcal meningitis. Since 2016, a laboratory-based reflex screening programme in South Africa automatically tests all individuals with CD4 plasma/serum samples <100 cells/µl for cryptococcal antigen (CrAg). Although 92% of eligible people have been screened, it is unclear whether cryptococcal meningitis incidence and mortality have reduced. We aimed to investigate barriers to screening implementation and propose opportunities for improvement. Methods We conducted 21 focus group discussions and 112 questionnaires with clinic staff in 4 provinces to explore their knowledge of CA and barriers to care for patients following a positive result. We conducted interviews with 14 investigators of a CA treatment trial (EFFECT, ISRCTN 30579828) to explore its impact on patient care. Results Clinic staff described fragmented, poorly defined pathways to care for patients following a positive reflex CrAg test. Key barriers included lack of standardised result notification systems, unclear clinical ownership of results, difficulty tracing and referring patients for lumbar puncture, and limited confidence in CA management. Consequently, positive results were frequently missed or acted upon only weeks to months later. EFFECT investigators described potential solutions including creation of regional roles dedicated to linking patients to care following a positive reflex CrAg test and using text messaging to improve notification systems. Conclusions Insights from clinic staff and EFFECT investigators could be used to inform interventions that improve the impact of CA screening in South Africa.

162 – Neuropathological differences driven by cryptococcal species in a murine model of infection

Maahir Kauchali

Emily Ruth Higgit 1, 2, 3, Yanga Pato 1, 2, 3, Jennifer Claire Hoving 3, 4, and Rachael Dangarembizi 1, 2, 3

1Division of Physiological Sciences, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, South Africa. 2Neuroscience Institute, Faculty of Health Sciences, University of Cape Town, Groote Schuur Hospital, South Africa 3CMM AFRICA Medical Mycology Research Unit, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, South Africa

Cryptococcal meningitis (CM) is a fatal brain infection associated with debilitating neurological sequelae. CM research has historically focused on understanding the neuropathology of Cryptococcus neoformans-related infections, neglecting C. gattii -related CM. Recent rises in C. gattii-related CM, coupled with the fact that it predominantly affects immunocompetent individuals, highlights a dire need for experimental models for studying its neuropathogenesis. This study aimed to optimize a model of C. gattii infection and compare its neuropathogenesis to C. neoformans. We intravenously infected 8–10-week-old C57BL/6 mice with 5x104 colony forming units of C. gattii and C. neoformans. We monitored disease progression by measuring fungal burden, body mass gain and survival rates. Histological analysis was performed on 1, 3, 6 days post-infection (dpi) and experimental endpoint assessing neuropathological damage. Brain and serum cytokines were measured using multiplex assays. We observed marked differences in the neuropathogenesis of C. neoformans– and C. gattii–associated CM. C. neoformans infection progressed rapidly, mice succumbed within a week post-infection, compared to nearly one month for C. gattii. Despite longer incubation periods observed during C. gattii infection, end-stage disease in both models was characterized by comparable fungal burdens. Large cryptococcal lesions were evident as early as 6 d.p.i in C. neoformans-infected mice, while C. gattii was associated with smaller lesions. Cytokine analysis further revealed significant species-specific differences in inflammatory profiles across d.p.i. These findings highlight distinct temporal and pathological features of C. neoformans and C. gattii infection and support the utility of this model for investigating C. gattii–induced CM.

163 – Defining the Cryptococcus neoformans titanide, a pathogenic morphotype that arises from typical yeast cells in response to host-relevant conditions

Abigail Potton

Iana Kalinina, Sandra Catania, Maria Makarova, Dora Corzo-Leon, Elizabeth Ballou

MRC Centre for Medical Mycology, University of Exeter

Fungal morphogenesis is a major driver of disease outcome. For the opportunistic fungal pathogen Cryptococcus neoformans, morphological heterogeneity within the lung drives dissemination, immune evasion, and drug resistance. To better understand how this heterogeneity emerges, we used microfluidics to study C. neoformans under host-mimicking conditions. We observed that yeast cells are highly plastic and respond by propagating as typical yeast (5-7 m), transitioning into titan cells (>10 m), or by generating an alternative daughter, the oval titanide (2-3 m). Titanides appear 24 hours post-induction and by 72 hours are the dominant morphotype. Despite their prevalence and the significance of morphogenesis for virulence, until now, their biology remained unstudied. Using TEM and fluorescence microscopy we demonstrate that titanides are morphologically distinct; they possess thin cell walls and capsules with reduced Pathogen Associated Molecular Pattern exposure and altered distribution. These features distinguish titanides from previously described C. neoformans small cells such as in vivo seed cells (4-6 m, high cell-wall mannan content) and micro cells (round, <1 m, thick cell walled). Despite their thin cell walls, we show that titanides display increased resistance to cell wall/membrane stressors. Additionally, titanides have unique proliferation properties and a morphological plasticity that allows them to form yeast and titan cells in response to relevant culture conditions. Importantly, we demonstrate that titanides are an efficient infectious propagule in a murine model with equivalent pathogenicity to infection with yeast. Together, these findings establish an essential characterisation and insight into a key player in C. neoformans pathogenesis.

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