53rd BSMM Annual Scientific Meeting

St John's Hotel, Solihull, Birmingham
March 19th -21st 2017

Meeting Programme

All sessions will take place in the Brueton Room, Park Suite

Posters will be displayed in the Malvern Room, Park Suite

SUNDAY 19TH MARCH 2017

From 13.30	**Registration (Park Suite Entrance)**
14.00 -16.00	BSMM Executive Committee Meeting (**Room tbc**) (*BSMM Executive Only*)
14.30 – 16.30	**WTSA Early Careers Workshop (*Open to all*)** Chaired by Simon Johnston *(University of Sheffield)* Tanya Roberts *(Senior Medical Scientist, Gilead),* Tim Dafforn *(Chief Entrepreneurial Advisor, BEIS, UK Government)* Georgina Drury (Research Support Partner, University of Birmingham) Sheba Agarwal-Jans, *(Journal Publisher, Elsevier),* David Brown *(Biosciences Technical Lead, Health & Safety Executive)* Tehmina Amin *(Project Administrator, Wellcome Trust Strategic Award)* Andy Borman *(Principal Clinical Scientist, Public Health England)*

SESSION ONE

CHAIR: LEWIS WHITE (PUBLIC HEALTH WALES)

16.45	**Official Welcome**
17.00	Poster Elevator Talks (6 x 5min slots) *Leenah Alaalm, Jagdish Chander, Kangzhen Dong, Robert Evans, Mariam Garelnabi, Jemima Ho*
17.30	**BSMM Foundation Lecture, Gordon Brown** *(MRC Centre for Medical Mycology, Aberdeen)* Chaired by Tom Rogers *(Trinity College Dublin)*
18.30	**Poster Session 1**
19.30	**Dinner & Late Bar**

MONDAY 20TH MARCH 2017

SESSION TWO

CHAIR: GORDON RAMAGE (DENTAL SCHOOL, GLASGOW)

09.00 **Keynote 1: Jessica Quintin**
(Institut Pasteur, Paris)

09.30 Offered Talk 1: Josie Gibson
***Cryptococcus neoformans* expansion in *in vivo* infection and the role of autophagy in host phagocyte cells**
(University of Sheffield & A-Star, Singapore)

09.45 Offered Talk 2: Margherita Bertuzzi
Single cell analysis of the *Aspergillus fumigatus* host-pathogen interaction at the alveolar epithelium
(University of Manchester)

10.00 Offered Talk 3: Neil Gow
Immune consequences of the spatial organization the *Candida* cell wall:
(MRC Centre for Medical Mycology, Aberdeen)

10.15 Offered Talk 4: Aleksandra Jasiulewicz
In vivo* phagocyte responses to the opportunistic fungal pathogen *Mucor circinelloides
(University of Birmingham)

10.30 Tea/Coffee

SESSION THREE

CHAIR: KERSTIN VOELZ (UNIVERSITY OF BIRMINGHAM)

11.00 **Keynote 2: Bill Steinbach**
(Duke University, USA)

11.30 Offered Talk 5: Andy Borman
***Candida auris* in the UK**
(National Mycology Reference Laboratory)

11.45 Offered Talk 6: Iain Page
Cut-offs for the Siemens Immulite *Aspergillus*-specific IgG assay for diagnosis of chronic pulmonary aspergillosis in Uganda
(University of Manchester)

12.00 Offered Talk 7: David Denning
Update on GAFFI Activities: Global Action Fund for Fungal infections, Switzerland and UK.
(University of Manchester)

12.15	**Gilead Fellows Talk 1: Adilia Warris,** *(MRC Centre for Medical Mycology, Aberdeen)*
12.30	Lunch

SESSION FOUR

CHAIR: GORDON BROWN (UNIVERSITY OF ABERDEEN)

13.30	**The Gilead Lecture: William Hope** *(Institute of Translational Medicine, Liverpool)*
14.00	Offered Talk 8: Catherine Pashley **The lung fungal microbiome in patients with asthma** *(University of Leicester)*
14.15	Offered Talk 9: Sergio Moreno-Velasquez **The dynamic changes in the β-1,3-Glucan synthase localization caused by caspofungin involve hyphal tip-lysis coupled with intrahyphal growth and paradoxical effect in *Aspergillus fumigatus*** *(University of Manchester)*
14.30	Poster Elevator Talks (6 x 5min slots) *Simon Johnston, Paula Seoane, Volha Skrahina, Wioleta Trzaska, Leighann Sherry, Alfred Kamuyango*
15.00	Tea/Coffee

SESSION FIVE

CHAIR: ADILIA WARRIS (UNIVERSITY OF ABERDEEN)

15.30	**Keynote 3: Anne Puel** *(INSERM, Paris)*
16.00	**Gilead Fellows Talk 2: Darius Armstrong-James** *(NHLI, Imperial College)*
16.15	AGM (*All BSMM Members Welcome*)
17-17.45	MSc Mycology Meeting *(By Invitation Only)*
17-18.30	**Poster Session 2**
19.15- late	**Dinner, Dancing, Sing-along**

TUESDAY 21ST MARCH 2017

SESSION SIX

CHAIR: NEIL GOW (UNIVERSITY OF ABERDEEN)

09.30 **Keynote 4: Bernhard Hube**
(HKI Jena)

10.00 Offered Talk 10: Peter Cook
Dendritic cells are required to mediate and sustain type-2 and type-17 allergic inflammation against *Aspergillus fumigatus*
(University of Manchester)

10.15 **The Basilea Lecture: Ashraf Ibrahim**
(Los Angeles Biomedical Research Institute, UCLA)

10.45 *Tea/Coffee*

SESSION SEVEN

CHAIR: JULIAN NAGLIK (KINGS COLLEGE LONDON)

11.15 **Keynote 5: Duncan Wilson**
(MRC Centre for Medical Mycology, Aberdeen)

11.45 Offered Talk 11: Daniel Fischer
***Candida glabrata* persistence within macrophages**
(HKI, Jena)

12.00 Offered Talk 12: Rebecca Hall
Adaptation of *Candida albicans* to environmental pH induces cell wall remodelling and enhances innate immune recognition
(University of Birmingham)

12.15 Offered Talk 13: Selinda Orr
IL-27 Induced by Select *Candida* Spp. via TLR7/NOD2 Signalling and IFN-β Production Inhibits Fungal Clearance
(Cardiff University)

12.30 **Gilead Fellows Talk 3: Gordon Ramage**
(Dental School, Glasgow)

12.45 Closing Address

12.50 Lunch

14.00 End of Conference

SESSION ONE

CHAIR: LEWIS WHITE (PUBLIC HEALTH WALES)

Elevator Abstracts

Understanding How the Hsp90 Kinase Interactome Affects *Candida albicans* Virulence

Leenah Alaalm, Carolyn Williamson, Kangzhen Dong, Ranjith Rajendran, Gordon Ramage, Stephanie Diezmann
Department of Biology & Biochemistry, The Milner Centre for Evolution, University of Bath, Claverton Down, BA2 7AY, Bath, UK.
Glasgow Dental Hospital & School, University of Glasgow Dental School , 378 Sauchiehall Street, Glasgow, G2 3JZ, UK.

Systemic infections caused by *Candida albicans* are often life-threatening and difficult to treat due to therapeutic limitations. Here, we are focusing on the essential and central regulator, the heat shock protein 90 (Hsp90) hence it plays a key role in regulating several virulence traits of *C. albicans.* Yet, treatment combining standard antifungal therapy and Hsp90 inhibition resulted in severe host toxicity in the murine model of systemic candidiasis. Thus, novel drug targets are urgently needed. A chemical genomic screen revealed that Hsp90 regulates 226 *C. albicans* genes, with 35 encoding kinases, which are considered attractive drug targets. We propose that combinatorial therapy comprising Hsp90 inhibitors, which are already in clinical trials as anti-cancer drugs, and *C. albicans*-specific kinase inhibitors could provide an effective therapeutic strategy for the treatment of systemic candidiasis. To identify suitable kinases in *C. albicans*, we study two sets, those that regulate Hsp90 function (Ck2 kinase complex) and those that require Hsp90 for proper function (Hsp90-target kinases). So far, we identified novel physical interactions between Hsp90 and its kinase-targeting co-chaperone Cdc37 and the Ck2 kinase complex. We selected nine Hsp90-target kinases, four highly conserved and five uncharacterized, fungal-specific kinases for further study of their virulence potential. Gene deletion mutants are characterized in a series of *in vitro* and *in vivo* assays. So far, we identified three kinases affecting morphogenesis, and two kinases regulating Fluconazole resistance in an Hsp90-dependent fashion. Understanding how the Hsp90-kinase circuitry regulates virulence will be a crucial milestone in our efforts to identify novel drug targets.

Prevalence of mucormycosis over five years' period from a north Indian tertiary care centre

[1]**Jagdish Chander**, [1]Nidhi Singla, [1]Mandeep Kaur, [1]Neelam Gulati, [2]RPS Punia, [3]SK Singhal, [4]Deepak Aggarwal, [5]Ashok Kumar Attri.

Departments of [1]Microbiology, [2]Pathology, [3]ENT, [4]Pulmonary Medicine and [5]Surgery, Government Medical College Hospital, Chandigarh (India)

Introduction: Mucormycosis is one of the emerging opportunistic fungal infections. Molecular phylogenetic analysis has led to class name Zygomycota being replaced by Glomeromycota and under this new classification, all the agents of mucormycosis have been placed under the subphylum Mucoromycotina. Increasing immunocompromised situations, widespread use of antibacterial and antifungal agents (like voriconazole prophylaxis), carcinomas and transplantation as well as lifestyle disease like diabetes mellitus contribute primarily to this situation. The predominant clinical manifestations of mucormycosis vary from host to host with rhino- orbital-cerebral, pulmonary, cutaneous, gastrointestinal being the main presenting clinical conditions. In India, the prevalence of mucormycosis is approximately 0.14 cases/1000 population, which is about 70 times the worldwide-accepted rate.

Patients and Methods: The present study was undertaken over a period of five years (January 2011 - December 2015) to look for prevalence of mucormycosis. The samples suspected of mucormycosis were examined by direct KOH/CFW wet mount and cultured on Sabouraud's dextrose agar without actidione and on blood agar as per standard mycological techniques. Histopathological correlation was also done for most of the cases.

Results and Discussion: We identified a total of 82 cases of mucormycosis out of a total of 6365 samples received for mycological evaluation during the said time period. Out of these, 55 were male patients and 27 were females. Most common presentation was rhino-orbito- cerebral (37), followed by cutaneous (25), Pulmonary (14), oral cavity involvement (4) and gastrointestinal (2). The commonest risk factor was diabetes and I/M injections. The fungus isolated was *Rhizopus oryzae* (17), *Apophysomyces variabilis* (12), *R. microsporus* (9), *Lichtheimia ramosa* (8), *Saksenaea erythrospora* (5), *Syncephalastrum racemosus* (4), *R. homothallicus* (2), *Rhizomucor pusillus* (1), *Mucor irregularis* (1) and *A. elegans* (1). Mainstay of the treatment was amphotericin B along with extensive surgical debridement whenever feasible. Most of the patients 50, recovered but 25 died. Rest of the patients left against medical advice.

Conclusion: 'Nip in the Bud' should be the mantra for clinicians / surgeons for a favorable prognosis. Early diagnosis, prompt institution of appropriate antifungal therapy, surgical debridement whenever necessary, knowledge of risk factors and their timely reversal is the key for management.

Hsp90 capacitates phenotypic variation via Loss-of-Heterozygosity and aneuploidy in *Candida albicans*

Kangzhen Dong[1], Leenah Alaalm[1], Steve W Milne[1], Heath O'Brien[2], Darren Abbey[3], Judy Berman[4], Anja Forche[5], Stephanie Diezmann[1]

1. Department of Biology & Biochemistry, the Milner Centre for Evolution, University of Bath, Bath, United Kingdom, s.diezmann@bath.ac.uk ; 2. MRC Centre for Neuropsychiatric Genetics & Genomics, Division of Psychological Medicine & Clinical Neurosciences, Cardiff University School of Medicine, OBrienH1@cardiff.ac.uk ; 3. Department of Genetics, Cell Biology and Development, University of Minnesota Twin Cities; 4. Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel, Jberman@post.tau.ac.il ; 5. Department of Biology, Bowdoin College, 6500 College Station, Brunswick, ME 04011, USA, aforche@bowdoin.edu

Candida albicans, the leading fungal pathogen of humans, does not appear to engage in canonical sexual recombination, which raises the question: how does this 'obligate' diploid generate genetic variations? Here, we address this question by investigating if and how Hsp90, a central regulator of protein homeostasis that governs virulence and drug resistance in *C. albicans*, regulates genome integrity in *C. albicans*. Our work demonstrated that different modes of Hsp90 perturbation increase Loss-of-Heterozygosity (LoH) rates across the *C. albicans* genome and induce aneuploidy, suggesting that Hsp90 is required for faithful maintenance of the diploid, heterozygous genome. Compromising Hsp90 function promotes genomic rearrangements leading to phenotypic variation. More importantly, our SNP-RFLP analyses revealed that the types of LoH events are contingent on the mechanisms used to reduce Hsp90 function. Pharmacological inhibition increased frequency of local recombination events such as gene conversion or break-induced recombination, whereas high temperature stress caused whole-chromosome homozygosis. This novel observation suggests that *C. albicans* may utilize distinct forms of LoH to deal with disparate environmental stressors. This concept is supported by our *in vitro* fungal drug resistance tests that showed a biological relevant pattern. In summary, our work provides evidence that Hsp90 governing LoH and aneuploidy facilitates *C. albicans* genome plasticity, which aids in the generation of novel genotypes and phenotypes. This indicates that Hsp90 affects a pathogen's virulence through genome changes, impacting both theoretical and clinic knowledge.

Fungal derived 15-keto-prostaglandin E_2 and host proliferator-activated receptor gamma (PPAR-) promote *C. neoformans* growth during infection.

Robert J. Evans[1,2], Sarah Needs [3], Ewa Bielska[3], Robin C. May[3,4], Stephen A. Renshaw[1,2], Simon A. Johnston[1,2]

[1] Bateson Centre, Firth Court, University of Sheffield, S10 2TN, UK. [2] Department of Infection, Immunity and Cardiovascular Disease, Medical School, University of Sheffield, S10 2RX, UK. [3] Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK. [4] NIHR Surgical Reconstruction and Microbiology Research Centre, University Hospitals of Birmingham NHS Foundation Trust, Queen Elizabeth Hospital, Birmingham, UK

Cryptococcus neoformans is one of the leading causes of invasive fungal infection in humans worldwide. *C. neoformans* can use macrophages as a proliferative niche to increase infective burden and avoid immune surveillance. However, the specific mechanisms by which *C. neoformans* manipulates host immunity to promote its growth

during infection remain ill-defined. Here we demonstrate a key role for eicosanoid lipid mediators produced by *C. neoformans* in regulating host responses.

Cryptococcus produces several eicosanoids that are similar to vertebrate eicosanoids, it is therefore possible that fungal-derived eicosanoids may mimic physiological effects in the host. Using a combination of *in vitro* and *in vivo* infection models we identify a specific eicosanoid species - prostaglandin E_2 – that is required by *C. neoformans* for growth during infection. Using the eicosanoid deficient cryptococcal D*plb1* mutant, we demonstrate that prostaglandin E_2 is required by *C. neoformans* for proliferation within macrophages and, using our zebrafish model of cryptococcosis, we confirm this role for PGE_2 *in vivo*. Furthermore, we show that PGE_2 must be dehydrogenated into 15-keto PGE_2 to promote fungal growth. Thus, we have shown for the first time that synthesis of eicosanoids by *C. neoformans* can directly promote intracellular growth within macrophages and *in vivo* within the host. Additionally, we have uncovered a new virulence factor – 15-keto-PGE_2 – that is produced from PGE_2 during infection. Finally, we also identify a new target for anti-fungal therapy by providing evidence that 15-keto PGE_2 mediates virulence via activation of host PPAR-g - an intracellular eicosanoid receptor that supresses protective immunity.

The role of host variability in determining macrophage responses to cryptococcosis

Mariam Garelnabi, Robin C. May

Institute of Microbiology & Infection and the School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Cryptococcosis is a fungal disease caused by the opportunistic pathogens *Cryptococcus neoformans* and *Cryptococcus gattii.* Although the majority of cryptococcosis cases occur in HIV patients, there is growing evidence to support the pathogenicity of *Cryptococcus spp.* towards otherwise healthy individuals. To date, however, few studies address how this fungal pathogen is able to evade host innate immune responses in individuals with more robust antifungal defenses.

We have been comparing primary immune responses to Cryptococcosis by monitoring the intracellular pathogenicity of cryptococci to host monocyte-derived macrophages – a critical cell type in anti-cryptococcal defense, from 15 healthy individuals. We have documented reproducible person-to-person variability in the rates of intracellular proliferation and vomocytosis, or non-lytic extrusion, of the pathogen; and aim to relate these effects to varying M1/M2 cytokine responses. Together, these data suggest that, even in immunocompetent individuals, intrinsic differences in macrophage responses may play an important role in regulating susceptibility to this fungal infection.

Candidalysin signals through the epidermal growth factor receptor (EGFR)

Jemima Ho[1], Spyridoula Nikou[1], Jonathan Richardson[1], David Moyes[1], Julian Naglik[1]

[1]*Mucosal and Salivary Biology Division, The Dental Institute, King's College London, UK.*

Background: *Candida* infections cause severe morbidity and mortality in millions each year and recent increases in incidence have been observed. Particularly susceptible are those that are immunocompromised due to existing infection or following immune therapy. Candidalysin is a newly identified *Candida albicans* derived toxin, which is critical for epithelial cell damage and mucosal pathogenicity[1]. Mechanisms of Candidalysin function are unclear.

Methods: TR146 human buccal epithelial cells were pre-treated with EGFR kinase inhibitors (Gefitinib or PD153035) 1 h prior to Candidalysin exposure. Protein expression was measured at various time points from cell lysate and culture supernatant samples.

Results: Stimulation of TR146 cells with Candidalysin, induced secretion of EGFR ligands (e.g. epiregulin and amphiregulin) and inflammatory cytokines (IL-1β, GM-CSF and G-CSF). Additionally, EGFR phosphorylation and expression of downstream signalling molecules, cFos and MKP1, were upregulated. Further, inhibition of EGFR kinase activity significantly suppressed Candidalysin-induced cFos, MPK1 and cytokine expression.

Conclusion: EGFR activation is critical for Candidalysin signaling and inflammatory cytokine responses in TR146 epithelial cells. Further investigation is required to understand the implications of cytokine signalling during infection, though induction of protective defences is a likely outcome. This knowledge may help determine the therapeutic value of EGFR and its downstream signalling pathways during *C. albicans* infections. Additionally, following Candidalysin exposure, released EGFR ligands may be responsible for activating EGFR, however direct Candidalysin-EGFR binding cannot be ruled out.

SESSION TWO

CHAIR: GORDON RAMAGE (DENTAL SCHOOL, GLASGOW)

Offered Talks

Cryptococcus neoformans expansion in *in vivo* infection and the role of autophagy in host phagocyte cells

Josie F. Gibson[1, 2, 3], Philip W. Ingham[1], Stephen A. Renshaw[2, 3] & Simon A. Johnston[2, 3]

1. Institute of Molecular and Cell Biology, Agency of Science, Technology and Research (A-Star), 61 Biopolis drive, Proteos, Singapore. 2. Department of Infection, Immunity and Cardiovascular Disease, Medical School, University of Sheffield, S10 2RX, UK
3. Bateson Centre, Firth Court, University of Sheffield, S10 2TN, UK.
Correspondence: jgibson4@shefield.ac.uk

Autophagy, a cellular self-degradation process degrades unwanted or damaged cellular components, including pathogens. Autophagy is implicated as a cellular niche for intracellular pathogens, able to subvert autophagy for their own end. *Cryptococcus neoformans* can survive within host phagocytes, predominantly macrophages. Interestingly, *C. neoformans* infection has been associated with a requirement for host

cell autophagy for increased cryptococcal replication. The route of cryptococcal infection is through air dispersal and deposition in the lung, however the fungal tropism towards the brain and CNS is not fully understood, here we investigate clonal expansion in terms of infection propagation.

The zebrafish model enables live *in vivo* cellular and sub-cellular imaging, this, coupled with transgenic and mutant lines, provides a valuable model for study of host-cryptococcal interactions. The aims of this project are two-fold. First, zebrafish autophagy mutants and transgenic lines are used to study autophagy in cryptococcal infection and visualise autophagic interactions within infected host phagocytes. Second, to investigate *in vivo* cryptococcal expansion and influence on the outcome of infection.

Involvement of autophagy in cryptococcal infection was confirmed through association of cryptococci with autophagic machinery. Zebrafish deficient in key autophagy genes, ATG3 and ATG5, which interestingly exhibit a survival defect, were infected and then mortality and association with autophagy marker LC3-II examined. Expansion of cryptococci was studied through mixed inoculum of GFP:mCherry, monitored until systemic. It was observed that a single cryptococci was not responsible for systemic infection and that there is an association between blood vessel size and likelihood of clonal expansion.

Single cell analysis of the *Aspergillus fumigatus* host-pathogen interaction at the alveolar epithelium

Margherita Bertuzzi[1], Gareth Howell[2], Lea Gregson[1], Viktorija Makarovaite[1], Mirac Demirbag[1] and Elaine M. Bignell[1]

[1]Manchester Fungal Infection Group, University of Manchester, Manchester, UK, margherita.bertuzzi@manchester.ac.uk; [2]Manchester Collaborative Centre for Inflammation Research, University of Manchester, Manchester,

A hallmark of invasive lung diseases caused by *Aspergillus fumigatus* is the penetrative growth of fungal hyphae across the respiratory epithelium. Previously we exploited *in vitro* alveolar infection assays to address the mechanistic basis of pathogen-mediated tissue damage, finding that *A. fumigatus* causes epithelial decay in a multiphasic fashion. Non-invasive and attenuated mutants lacking the transcription factor PacC are unable to cause epithelial decay. Indirect quantification via nystatin protection assay indicated that *ΔpacC* mutants are internalised less efficiently by epithelial cells (ECs) relative to the respective parental isolates. The pathological relevance of spore internalisation remains unknown. The focus of this study was therefore to establish a direct and single cell approach based on imaging flow cytometry to identify, quantify and isolate individual host pathogen complexes from infected ECs in order to understand the role of spore internalisation in disease outcome.

To assess the timing and stoichiometry of spore-ECs interactions and the rate of spore internalisation by A549 alveolar epithelia, a differential fluorescence assay was developed using Calcofluor White staining and a panel of tdTomato-expressing fluorescent *A. fumigatus* isolates. Uptake of *A. fumigatus* spores was rapid and time-dependent. Single epithelial cells were able to internalise multiple *A. fumigatus* spores. Remarkably spores internalised by epithelial cells were delayed for germinative growth. Relative to the parental isolate and in agreement with the nystatin protection assay, the non-invasive and attenuated *ΔpacC* mutant was less efficiently internalised. To survey the fate of host and pathogen cells respectively, the optimized multiplex flow cytometric assay was expanded to include Annexin V/TO-PRO staining and viability counts of internalised *A. fumigatus* spores. While viability of internalizing ECs was largely unaffected, only 1% of internalised spores remained viable after 8 hours of co-incubation.

Applying the same single cell approach to infected mouse lungs we have also optimised the isolation and typing of pathogen-interacting respiratory ECs revealing, for the first time in whole animals, that spores are internalised by type II ECs. The integration of the

data obtained demonstrates that uptake and killing of fungal spores by cultured human, and primary murine type II alveolar ECs can provide a potent means of pathogen control. The single cell approach presented has high potential to investigate the complexity of the host-pathogen interaction in health and disease.

Immune consequences of the spatial organization the Candida cell wall

Neil A.R. Gow

Institute of Medical Sciences, University of Aberdeen, Aberdeen AB252ZD, UK;
n.gow@abdn.ac.uk

The fungal cell wall defines the perimeter of its own self and a boundary of communication with other organisms with which it interacts. For a fungal pathogen, every component and layer of the cell wall plays a specific role in its interactions with cells of the human host and in resisting assault by enzymes, phagocytes and certain antibiotics. We have tried to unpeel the functions of cell wall components by combining structural analyses, genetics, biochemistry and immunology to understand how the wall is assembled and how its constituents are recognized by the human immune system. This work has led to a new model of the cell wall and a description of how the polysaccharides of the cell wall activate both the inflammatory and anti-inflammatory responses of the innate immune system.

In vivo phagocyte responses to the opportunistic fungal pathogen *Mucor circinelloides*

Aleksandra Jasiulewicz and Kerstin Voelz

Institute of Microbiology & Infection, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.
jasiulea@bham.ac.uk

Mucormycosis is a life-threatening infection, mostly affecting immunosuppressed individuals, with up to 95% mortality in disseminated cases. The innate immunity and particularly phagocytic effector cells are the host's first line of defense against infectious mucormycete spores. Yet, we currently have limited knowledge on the *in vivo* dynamics of the phagocyte response during mucormycosis.

We employed a zebrafish larval *in vivo* model to determine the spatiotemporal patterns of innate immunity to fungal spores in healthy and immunocompromised hosts. We mimicked immunosuppressive conditions in 36 hours old transgenic zebrafish embryos with fluorescently labeled macrophages and neutrophils by dexamethasone treatment 4 hours before microinjection with *Mucor circinelloides* spores in the hindbrain ventricle. Phagocyte recruitment to the site of infection was assessed using fluorescence microscopy and related to larval survival and spore killing.

Spore injection initiated rapid recruitment of neutrophils and macrophages to the site of infection forming tight granulomatous clusters around the infectious particle in healthy hosts. This early granuloma formation protects from spore dissemination and onset of disease. Phagocyte recruitment was impaired in dexamethasone-treated larvae, which showed reduced granuloma formation and increased dissemination leading to hyphal growth and ultimately a fatal outcome.

In summary, this is the first report on the spatiotemporal dynamics of phagocyte recruitment in response to mucormycete spores. We demonstrate that early recruitment of phagocytes protects from disease by controlling fungal spores in nascent granulomas. In immunocompromised hosts this recruitment fails leading to spore dissemination and disease.

SESSION THREE

CHAIR: KERSTIN VOELZ (UNIVERSITY OF BIRMINGHAM)

Offered Talks

Candida auris in the UK

Andrew M. Borman, Adrien Szekely, Michael D. Palmer and Elizabeth M. Johnson

Public Health England UK National Mycology Reference Laboratory, Myrtle Road, Bristol BS2 8EL, UK Andy.Borman@uhBristol.nhs.uk

Since its first description from discharge from a human external ear canal in japan in 2009, *Candida auris*, a novel *Candida* species in the *Candida haemulonii* complex, has emerged as a serious nosocomial health risk, with widespread outbreaks in numerous hospitals worldwide and the existence of geographic region-specific discrete clonal lineages. The first 3 UK isolates of *C. auris* were received at the UK National Mycology Reference Laboratory (MRL) in 2013, from blood cultures from 3 unrelated patients in distant geographical localities. Since 2013, we have received a further 160 isolates from at least 14 different UK hospitals, including a number of isolates suspected of being part of several large local outbreaks. rDNA sequence comparisons of *Candida auris* isolates from different hospital centers in the UK with those of strains from different international origins present in the public sequence databases revealed that UK isolates of *C. auris* fall into three well supported clades corresponding to lineages that have previously been reported from India, Malaysia and Kuwait, Japan and Korea, and South Africa, respectively, with clear evidence of multiple independent introductions of different *C. auris* strains in several centers. Here, we present the results of rDNA sequence analyses, and data that demonstrate that the various clonal lineages circulating in the UK differ in terms both of pathogenic potential/virulence in an invertebrate model of infection and *in vitro* antifungal susceptibility to the triazole antifungals.

Cut-offs for the Siemens Immulite *Aspergillus*-specific IgG assay for diagnosis of chronic pulmonary aspergillosis in Uganda

Iain Page[1], Malcolm Richardson[1],[2, 3] & David Denning[1,2]

1 –NATIONAL ASPERGILLOSIS CENTER, UNIVERSITY HOSPITAL OF SOUTH MANCHESTER, UK.

2 -DIVISION OF INFECTION, IMMUNITY AND RESPIRATORY MEDICINE, FACULTY OF BIOLOGY, MEDICINE AND HEALTH, MANCHESTER ACADEMIC HEALTH SCIENCE CENTRE, THE UNIVERSITY OF MANCHESTER, UK.

3 - MYCOLOGY REFERENCE CENTRE MANCHESTER, UNIVERSITY HOSPITAL OF SOUTH MANCHESTER, UK.

iain.page@manchester.ac.uk

We recently published a proposed optimal cut-off of 10 mg/L for the Siemens Immulite *Aspergillus*-specific IgG assay, using ROC analysis comparing healthy Ugandan controls (median age 19 years) to CPA cases(1). We now present further ROC analyses with two groups of older Ugandan controls.

100 sera from older healthy Ugandan blood donors and 88 diseased Ugandan controls with previously treated tuberculosis, but without symptoms or radiological features of CPA were tested by Siemens Immulite *Aspergillus*-specific IgG assay. ROC analysis was

performed comparing results in these control groups to the same 241 sera from CPA cases analysed in the original paper. Optimal cut-offs were calculated by applying Youden's J statistic to co-ordinate points on the ROC results.

Median *Aspergillus*-specific IgG levels and optimal cut-offs are shown in the results table, together with the sensitivity, specificity and Youden's J statistic result for each cut-off in the relevant population. No significant correlation between results and patient age was found in any group.

Aspergillus-specific IgG levels are lower in children than adults. It is not known at what age adult levels are achieved. It is therefore possible that the cut-off derived using the original 100 controls (median age 19) is not optimal for use in an adult population due to the inclusion of many adolescents in the control group. Cut-offs derived using healthy and diseased controls are similar. We would suggest that the cut-off of 20mg/L, derived using the oldest 100 healthy controls is optimal for the purpose of CPA diagnosis in Ugandan adults.

Results table

Control Group	Median age	Age range	Median *Aspergillus*-specific IgG level	Optimal cut-off	Sensitivity	Specificity	Youden's J statistic
Original healthy Ugandan controls from 2016 paper N = 100	19	17 - 39	3.83 mg/L	10 mg/L	96%	98%	
Oldest healthy Ugandan controls N = 100	22	20 - 39	4.58 mg/L	20 mg/L	93%	98%	0.914
Asymptomatic diseased controls with prior TB N=88	38	16 - 77	3.72 mg/L	25 mg/L	92%	99%	0.914

Reference

1. Page ID, Richardson MD, Denning DW. Comparison of six Aspergillus-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). J Infect. 2016 Feb;72(2):240–9.

Update on GAFFI activities

David W. Denning on behalf of the GAFFI Board, Advisors and country Ambassadors

Global Action Fund for Fungal infections, Switzerland and UK. www.GAFFI.org

GAFFI's mission is to reduce death and suffering from fungal diseases worldwide. Set up in 2013, GAFFI has driven awareness among global health agencies and many medical staff. GAFFI launched a 10 year Roadmap in May 2015 entitled '95-95 by 2025'. With its educational partner based in the UK - LIFE-Worldwide - the burden of serious fungal diseases has been estimated in 68 countries including the UK (Pegorie M, J Infect 2017; 23:177). The lack of generic antifungal drug access and their variable cost has been documented for most countries around the world (Kneale M, J Antimicrob Chemother 2016; 71:3599). The case was successfully made in 2013 for amphotericin B and flucytosine to be added to the WHO Essential medicine List and an application is pending for itraconazole, voriconazole and natamycin 5% ophthalmic solution. GAFFI has applied for chromoblastomycosis to be listed as a Neglected Tropical Disease alongside mycetoma. GAFFI has also estimated how many lives could be saved by implementing diagnostic tests and making itraconazole, amphotericin B and flucytosine available for patients with life-threatening infections in AIDS – ramping up to 60% coverage by 2020 would save over a million lives (Denning DW. Phil Trans Roy Soc B 2016; 371: 20150468). GAFFI has also argued that provision of fungal diagnostics will contribute to controlling AMR by reducing unnecessary anti-bacterial usage (Denning DW, Emerg Infect Dis 2017;23:177).

SESSION FOUR

CHAIR: GORDON BROWN (UNIVERSITY OF ABERDEEN)

Offered Talks

The lung fungal microbiome in patients with asthma

Eva-maria Rick[1], Kerry F. Woolnough[2], Abbie Fairs[1], Jack Satchwell[1], Michelle Bourne[2], Michelle Craner[2], Pam Cairns[2], Susan M. Laundon[2], William R. Monteiro[2], Hetan S. Bhatt[2], Gill Elliott[2], Andrew J. Wardlaw[1,2]* & Catherine H. Pashley[1]* (*joint senior authors)

[1]Department of Infection, Immunity and Inflammation, Institute for Lung Health, University of Leicester, Leicester, LE1 7RH, UK, chp5@le.ac.uk [2]Respiratory NIHR Biomedical Research Unit, University Hospitals of Leicester, Leicester LE3 9QP, UK

Objective

Sensitisation to *Aspergillus fumigatus,* and isolation of filamentous fungi from sputa of asthmatics by culture, have been associated with reduced lung function. The lung fungal microbiome (mycobiome) may be underestimated due to the inherent insensitivity of culture. The objective of this study was to use amplicon-based high-throughput sequencing (HTS) to determine the lung mycobiome in patients with asthma and healthy controls.

Methods

Patients with asthma were classified into two groups; IgE-sensitised to any of a panel of six fungi and not fungal sensitised. A third group comprised healthy controls. All subjects provided sputum and a subset underwent bronchoscopy. DNA was extracted and the internal transcribed spacer region 2 of the fungal nuclear ribosomal operon amplified and subjected to paired-end sequencing on the Illumina MiSeq platform. Bioinformatic analysis was performed using QIIME.

Results

HTS data was obtained from 82 sputa, 67 from people with asthma of whom 41 were fungal sensitised. HTS data originating from a bronchoscopic sample was obtained from 18 patients.

Whilst more than 200 species were detected; only 14 species were present in > 50% of sputum samples, dominated by *A. fumigatus*, *A. niger, Candida albicans* and *Cladosporium* spp. The top five prevalent species were consistent between sputum, bronchial wash, bronchial brushings and bronchial lavage, although there was a trend towards greater species diversity in sputum.

Conclusions
HTS is a sensitive method to assess the lung mycobiome. The main fungal species detected were comparable between different patient groups and between different sample types.

The dynamic changes in the β-1,3-Glucan synthase localization caused by caspofungin involve hyphal tip-lysis coupled with intrahyphal growth and paradoxical effect in *Aspergillus fumigatus*

Sergio, D, Moreno-Velasquez[1], Constanze, Seidel[1], Praveen, R, Juvvadi[2], William, J, Steinbach[2], & Nick D. Read[1]

[1]*Manchester Fungal Infection Group, Division of Infection, Immunity and Respiratory Medicine, Faculty of Biology, Medicine and Health, University of Manchester, CTF Building, 46 Grafton Street, Manchester, M13 9NT, UK.* sergio.morenovelasquez@postgrad.manchester.ac.uk; dr.constanze.seidel@gmail.com; nick.read@manchester.ac.uk [2]*Division of Pediatric Infectious Diseases, Department of Pediatrics, Duke University Medical Center, Durham, USA. praveen.juvvadi@duke.edu; bill.steinbach@duke.edu*

Caspofungin targets the cell wall synthesizing enzyme β-1,3-glucan synthase and is used as second-line therapy to treat invasive aspergillosis. Despite its clinical importance, caspofungin exhibits attenuated activity at high concentrations, a phenomenon known as the 'paradoxical effect'. Here, we monitored the key morphological rearrangements due to impaired dynamics of the β-1,3-glucan synthase complex during caspofungin treatment by live-cell imaging in *A. fumigatus*. Treatment with either growth inhibitory (0.5 μg/ml) or paradoxical growth (4 μg/ml) concentrations of caspofungin for 24 h caused similar morphological defects including hyperbranching, abnormal septa distribution, and continuous hyphal tip-lysis. Notably, all lysed hyphae regenerated their extension by intrahyphal growth. Furthermore, the catalytic subunit of the β-1,3-glucan synthase, Fks1, was mislocalized from hyphal tips to vacuoles. This was concomitant with reduced β-1,3-glucan, but an increased α-1,3-glucan, galactomannan and chitin contents. However, continuous exposure to 4 μg/ml of caspofungin for 48 h led to normal hyphal morphology with the relocalization of Fks1 to hyphal tips, recovery of their normal α-and β-1,3-glucan, galactomannan contents and abolition of hyphal tip-lysis. Unexpectedly, hyphal tip localization of the regulatory subunit of the β-1,3-glucan synthase, Rho1, was unaltered upon caspofungin treatment but it was required for the paradoxical effect. Similarly, paradoxical effect was reproduced in an *ex vivo* human alveolar invasion model. Our results highlight the importance of intrahyphal growth as a rapid adaptation mechanism to the tip-fungicidal effect of caspofungin and the dynamic relocation of Fks1 as part of the recovery process for the induction of caspofungin-mediated paradoxical effect in *A. fumigatus*.

Elevator Abstracts

The T cell anti-proliferative mycophenolate mofetil increases susceptibility to cryptococcosis through inducing macrophage cell death and lysis.

Rory H. Gibson[1,2] *, Aleks Bojarczuk[1,2] *, Richard Hotham[1,2], Amy Lewis[1,2], Ewa Bielska[3], Robin C. May[3,4], Phil M. Elks[1,2], Stephen A. Renshaw[1,2] and Simon A. Johnston[1,2]

[1] Bateson Centre, Firth Court, University of Sheffield, S10 2TN, UK. [2] Department of Infection, Immunity and Cardiovascular Disease, Medical School, University of Sheffield, S10 2RX, UK. [3] Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK. [4] NIHR Surgical Reconstruction and Microbiology Research Centre, University Hospitals of Birmingham NHS Foundation Trust, Queen Elizabeth Hospital, Birmingham, UK
Correspondence: s.a.johnston@sheffield.ac.uk

Systemic cryptococcosis is associated with the severely immunocompromised, exemplified by its incidence in HIV co-infection where CD4 counts are <100 cell/mm^3. Other immune pathologies associated with increased susceptibility to cryptococcosis are much less understood, for example, Fc receptor 3A polymorphism and pulmonary alveolar proteinosis. Here we describe a mechanism for the increased susceptibility to cryptococcosis with the immunosuppressive anti-proliferative drug mycophenolate mofetil (MMF). MMF is a specific inhibitor of inosine monophosphate dehydrogenase that preferentially targets T and B cell purine synthesis due to an absence of a separate salvage pathway present in other cell types.

Using our zebrafish model of cryptococcosis we find that treatment with MMF, in the absence of adaptive immunity, results in a dramatic increase in fungal burden. We demonstrate that MMF causes macrophage cell death and increased neutrophil apoptosis following inflammatory insult. Comparison of MMF mechanism of action to azathioprine (a related drug) suggested a difference in cellular GTP depletion. This resulted in reduced macrophage numbers within the first 24 hours of infection, which were associated with lower phagocytosis independent of capsule-mediated inhibition. Linear regression analysis identified an additive association between total fungal burden and extracellular cryptococci. Using live time lapse imaging and we found that release of cryptococci due macrophage lysis was responsible for the increased fungal burden. Finally, we propose that loss of early control of cryptococcosis modeled here may have significant consequence for control and clearance of pulmonary infection during MMF treatment.

Exploring the effects of interferon alpha on *Cryptococcus neoformans* infection

Paula I., Seoane, Taylor-Smith, Leanne & May, Robin C.

Institute of Microbiology and Infection, University of Birmingham, UK
e-mail: PIS528@bham.ac.uk

Cryptococcus neoformans is an opportunistic human pathogen, which causes serious disease in immunocompromised hosts, in particular those with HIV. A key feature of cryptococcal pathogenesis is the ability of the fungi to survive and replicate within the phagosome of macrophages, as well as its ability to escape by a novel non-lytic mechanism known as vomocytosis. The impact of vomocytosis on the outcome of infection is currently unknown, although it has been proposed to be linked to dissemination.

We have been exploring the impact of inflammatory cytokine signalling on vomocytosis. Here we show that elevated levels of interferon alpha (IFN α), corresponding to those produced as a result of the anti-viral immune response during HIV infection, alter cryptococcal vomocytosis rates. Consequently, acute viral infection may trigger the release of latent cryptococci from intracellular compartments, with significant consequences for disease progression.

Candida albicans and trace metal

Volha Skrahina[1]*, Sascha Brunke[1,2], Jörg Linde[4], Bernhard Hube [1,2,3]

[1]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), Jena, Germany; [2]Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany; [3]Friedrich Schiller University, Jena, Germany; [4]Department of Systems Biology and Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), Jena, Germany

Zinc serves both as a structural and catalytic cofactor in a broad range of enzymes and is required for a multitude of biological processes. Thus, the availability of zinc is also recognized as a central factor in infections. For example, zinc acquisition and homeostasis are crucial for *Candida albicans*, a yeast, that co-exists as a harmless commensal in humans, but is also able to cause severe systemic infections, when the host's barriers are damaged or it's immune system is compromised.

We are investigating the response of *C. albicans* to zinc limitation (ZL). To this end, we deprived *C. albicans* of zinc *in vitro* and performed transcriptome analyses. As a hallmark of successful starvation, we found the 'zincophore' zinc acquisition system, consisting of secreted Pra1 and the zinc transporter Zrt1, which were strongly up-regulated. A mutant, lacking the central zinc importer Zrt2, showed a severe growth defect under ZL and up-regulated zinc uptake machinery even in the presence of zinc, indicating continuous intracellular zinc starvation.

To analyse the zinc homeostasis network in detail, 165 transcription factor (TF) mutants (Homann *et al.,* PLoS Genet., 2009) were grown under ZL. One of the mutants, *ssn6*Δ, exhibited a severe growth defect and had reduced transcript levels of *ZRT2* and the vacuolar zinc exporter gene, *ZRT3.* In contrast, *CSR1* level, encoding a TF essential for zinc up-take regulation, was increased, indicating a potential interaction of Ssn6 with Csr1. Therefore, our results show Ssn6 to be a likely new component in the fungal zinc homeostasis machinery.

Species-specific antifungal activity of blue light

Wioleta J. Trzaska, Helen E. Wrigley, Joanne E. Thwaite and Robin C. May

Institute of Microbiology and Infection and School of Biosciences, University of Birmingham, Edgbaston, Birmingham. B15 2TT, UK. NIHR Surgical Reconstruction and Microbiology Research Centre, Queen Elizabeth Hospital, Birmingham, U.K. Chemical, Biological and Radiological Division, DSTL, Porton Down, Salisbury, Wiltshire, UK; r.c.may@bham.ac.uk

Trauma-associated fungal infections represent a significant threat to immunocompetent and immunocompromised patients. Consequently, strategies to remove fungal pathogens from hospital surfaces or patient skin offer the potential for significant therapeutic benefit, in particular for patients suffering from traumatic injury. Recently, there has been considerable interest in using light to inactivate microbial pathogens, with data from several groups demonstrating that exposing bacterial, fungal and viral pathogens to different light wavelengths combined with photosensitizing dyes can effectively inactivate them.

Here we explore the antifungal potential of blue light, which appears to be effective against bacterial pathogens without the need to introduce exogenous photosensitizers into the pathogen. By testing a wide range of fungal pathogens, we show that blue light is effective against some but not all species. In addition, we highlight the potential for secondary heating to be a confounding factor when assessing the antimicrobial activity of blue light in some instruments.

Thus, blue light offers a potentially powerful antimicrobial approach, but its application in situations where infections with Mucormycetes are likely should be undertaken with caution.

Candida auris is highly virulent and can form biofilms: a new level of resistance

Leighann Sherry[1], Gordon Ramage[1], Ryan Kean[1], Andrew Borman[2], Elizabeth M Johnson[2], Malcolm D Richardson[3,4,5] and Riina Rautemaa-Richardson[3,4,5]

[1]Oral Sciences Research Group, School of Medicine, Dentistry and Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK; [2]Mycology Reference Laboratory, Public Health England, Bristol, UK; [3]Mycology Reference Centre Manchester, University Hospital of South Manchester, Manchester, UK; [4]The University of Manchester, Manchester Academic Health Science Centre, Faculty of Biology, Medicine and Health, Division of Infection, Immunity and Respiratory Medicine, Manchester, UK; [5]National Aspergillosis Centre, University Hospital of South Manchester, Manchester, UK.

Candida auris is an emerging pathogen, first reported in 2009, and has attracted attention due to its reduced antifungal susceptibility. More recently, it has been detected in a UK ICU, where 20% of patients colonised developed candidaemia. The ability of *C. auris* to form biofilms and resist antimicrobial therapy and host responses has not been studied. Therefore, we assessed *C. auris* pathogenicity in the context of biofilm forming capacity, susceptibility to a panel of antimicrobials, and its virulence *in vivo*.

Candida albicans SC5314, *Candida glabrata* WT2001 and *Candida auris* M/67838 were used for minimum inhibitory concentration testing, for six antifungals in addition to chlorhexidine, following CLSI guidelines. Isolates were screened for biofilm formation,

and sessile susceptibility testing performed. The *G. mellonella* model was used to assess pathogenicity *in vivo*.

C. auris displayed intermediate biofilm formation, consisting predominately budding yeast and occasional pseudo-hyphae. Chlorhexidine was effective against *C. auris* biofilms unlike other antifungals, where >16 mg/L was required to kill the biofilm. Although *C. albicans* and *C. auris* had similar kill kinetics *in vivo*, *C. auris* infection achieved a 100% mortality rate more rapidly than *C. albicans*.

C. auris is able to form biofilms and resist antifungals that are active against its planktonic counterparts. These features not only contribute to its virulence, but also to its survival in hospital environments, increasing its ability to cause outbreaks. The results of the *in vivo* model mimic our clinical experience and highlight *C. auris* as a highly virulent species, equivalent to *C. albicans*.

Activation of Innate Immunity Leads to Clearance of *Cryptococcus neoformans* Infection in Zebrafish

Alfred A. Kamuyango[1], Simon J. Foster[3], Robin May[4] and Simon A. Johnston[1,2]

[1]Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Sheffield, UK; [2]Bateson Centre, Department of Biomedical Sciences, University of Sheffield, Sheffield, UK; [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK; [4]School of Biosciences and Institute of Microbiology & Infection, University of Birmingham, UK

Cryptococcus neoformans is a fungal pathogen of the severely immunocompromised with defective T-cell mediated immunity e.g. those individuals with HIV/AIDS. *C. neoformans* is acquired through inhalation into the lungs where it encounters innate immune cells including alveolar macrophages. Activation of macrophages is essential for the control of cryptococcal infection. M1 macrophages are more potent at inhibiting cryptococcal growth than M2 macrophages. However, in the absence of T-cell mediated immunity, activation of macrophages is disrupted and prevents the clearance of cryptococcal infection.

TLR signaling is required for generation of protective innate immune responses against microbial pathogens. In this study, we evaluated the effect of TLR ligands, including pathogen associated molecular patterns (PAMPs), i.e. *Staphylococcus aureus* peptidoglycan, lipopolysaccharide, and imiquimod, or pro-inflammatory cytokines such as IFNγ, on stimulation of antifungal innate immunity in a zebrafish *C. neoformans* infection model. In this model, immune-modulatory factors were intramuscularly co-injected with *C. neoformans* and their efficacy in activating immunity determined by measuring fungal burden, infection clearance and, in parallel, changes in macrophage behavior.

Here we showed that peptidoglycan locally activates innate immunity and thereby induce strong protection against *C. neoformans* infection, which was attributable to markedly reduced fungal burden and improved fungal clearance. Using chemically digested peptidoglycan to remove wall teichoic acid and/or mutants that do not produce wall teichoic acid (*tarO*) or lipoproteins (*lgt*), we established that protective effects of peptidoglycan require wall teichoic acid but not lipoproteins or lipoteichoic acid. Protection by *lgt* peptidoglycan showed increased recruitment of macrophages and rapid phagocytosis. The protective effects owing to other macrophage behaviours such as phagosomal pH, ROS, pro-inflammatory genes expression, and myD88 signalling were also evaluated. With these data we are able to place immune signaling, effector molecules and macrophage behavior in the context of disease progression while identifying potential new immunomodulatory targets.

Offered Talks

Dendritic cells are required to mediate and sustain type-2 and type-17 allergic inflammation against *Aspergillus fumigatus*

Peter Cook[1,2], Sheila Brown[1], Cecilia Forss[1], Freya R. Svedberg[1], David .W. Denning[3], Angela Simpson[4], Robert Niven[4], Michael J. Bromley[2], Irmgard Förster[5], Elaine M. Bignell[2] and Andrew S. MacDonald[1].

1. Manchester Collaborative Centre for In ammation Research (MCCIR), University of Manchester, UK; 2. Manchester Fungal Infection Group (MFIG), University of Manchester, UK; 3. National Aspergillus Centre, University Hospital of South Manchester & University of Manchester, UK; 4. North West Lung Centre, University Hospital of South Manchester & University of Manchester, UK; 5. Immunology and Environment, Life and Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany.

Dendritic cells (DCs) are essential for the initiation of type-2 and type-17 responses against several fungal pathogens. However, the role of DCs in mediating allergic inflammation upon exposure to the fungus *A. fumigatus* (*Af*), is poorly understood. Furthermore, the relative impact of type-2 and type-17 responses on the severity of allergic inflammation against *Af* is not yet clear. Utilizing cytokine reporter mice, we have found that repeatedly exposing mice to live *Af* spores elicits simultaneous type-eosinophilic/type-2 and neutrophilic/type-17 allergic inflammation in the airways. We have found that $CD4^+$ T cells, not innate lymphoid cells, are the dominant source of type-2 and type-17 cytokine source throughout the 3-week model. Analysis of DC populations has revealed that $CD11b^+$ cDC2, not $CD103^+$ cDC1, become the dominant DC subset in the lungs and draining lymph nodes over time. Repeated exposure of *Batf3*$^{-/-}$ mice (deficient in cDC1) to *Af* spores induced similar responses to WT mice, demonstrating that allergic inflammation against *Af* is independent of cDC1. We are currently investigating the importance of specific cDC2 subpopulations in mediating type-2/type-17 *Af* allergic inflammation in mouse models. Furthermore, to confirm these findings translate to human disease, we are profiling the key DC subsets and their activation status in the airways of fungal asthma patients. Ongoing work is assessing the key mechanism(s) that mouse and human DCs employ to mediate anti-*Af* allergic responses, to reveal novel targets for development of new therapeutic approaches.

Offered Talks

Candida glabrata persistence within macrophages

Daniel Fischer[1], Sascha Brunke[1], Bernhard Hube[1,2,3]

[1]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute Jena (HKI), Beutenbergstrasse 11a, 07745 Jena, Germany; [2]Friedrich Schiller University, Jena, Germany; [3]Integrated Research and Treatment Center, Sepsis und Sepsisfolgen, Center for Sepsis Control and Care (CSCC), Universitätsklinikum Jena, Germany. daniel.fischer@leibniz-hki.de

Candida glabrata is an opportunistic pathogenic yeast which can cause superficial and systemic infections mainly in immunocompromised patients. Recurring *C. glabrata* infections accompanied by acquisition of drug resistance have been reported (1), however, the reservoir remains elusive. *C. glabrata* yeasts were found associated with mononuclear cells for at least four weeks in a symptom-free mouse model of systemic candidiasis (2) and *C. glabrata* can survive and replicate within macrophages *in vitro* (3). We thus postulate that *C. glabrata* can persist within mammalian hosts by interaction with macrophages, dendritic cells and/or monocytes.

To study the long-term *C. glabrata*-macrophage interaction we established a co-incubation model. We found that under conditions largely mimicking the presumed conditions in the host, *C. glabrata* survives, albeit in a declining population, for days to weeks inside macrophages. These conditions differ from previously used experimental systems, where the intracellularly replicating yeasts lysed the host cells within three days. With our new model, we are now in the position to analyse the long-term interaction of persisting *C. glabrata* and their host cells, and close this important gap in our knowledge on fungal pathogenesis.

To identify yeast factors that are needed for persistence within macrophages, we are currently in the process of screening a *C. glabrata* deletion mutant collection (4) in a pool-based approach, flanked and complemented by transcriptional analyses of the yeast cells during prolonged interaction with phagocytes. In this approach, we aim to find fungal persistence factors that can potentially be targeted in a clinical setting.

References:

1) Singh-Babak SD *et al.* (2012) PLoS Pathog.
2) Jacobsen ID *et al.* (2010) Infect Immun.
3) Seider K *et al*. (2011) J Immunol
4) Schwarzmüller T *et al.* (2014) PLoS Pathog.

Adaptation of *Candida albicans* to environmental pH induces cell wall remodelling and enhances innate immune recognition

Sarah L. Sherrington[1], Eleanor Sorsby[1], Nabeel Mahtey[1], Megan D. Lenardon[2], Ian Brown[3], Elizabeth Ballou[2], Donna M. MacCallum[2] and Rebecca A. Hall[1]

[1]Institute of Microbiology and Infection, and School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK, B15 2TT; [2]MRC Centre for Medical Mycology, Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK, AB25 2ZD; [3]School of Biosciences, University of Kent, Canterbury, Kent, CT2 7NZ.

Candida albicans is able to proliferate in environments that vary dramatically in ambient pH, a trait required for colonising niches such as the stomach, vaginal mucosal and the GI tract. Here we show that growth in acidic environments involves cell wall remodelling which results in enhanced chitin and β-glucan exposure at the cell wall periphery. Enhanced chitin exposure resulted from reduced expression of the cell wall chitinase Cht2, via a Bcr1-Rim101 dependent signalling cascade, while increased β-glucan exposure was regulated via a non-canonical signalling pathway. Unmasking of the underlying immuno-stimulatory β-glucan resulted in enhanced innate immune recognition and proinflammatory cytokine production mediate through the C-type lectin-like receptor, Dectin-1. This in turn resulted in significantly more innate immune cell recruitment to the site of infection *in vivo*. We propose that this "unmasking" of the cell wall may contribute to the non-protective hyperactivation of the innate immune system during growth in the acidic environment of the female reproductive tract resulting in symptomatic vaginal infection.

IL-27 Induced by Select *Candida* Spp. via TLR7/NOD2 Signalling and IFN-β Production Inhibits Fungal Clearance

Emmanuel C. Patin[1], Adam V. Jones[2], Aiysha Thompson[1], Gareth W. Jones[1] & Selinda J. Orr[1]

[1]Division of Infection & Immunity, Cardiff University School of Medicine, Cardiff, CF14 4XN; [2]University Dental Hospital, Cardiff and Vale University Health Board, Cardiff, CF14 4XY.

Introduction: *Candida* spp. elicit cytokine production downstream of various pathogen recognition receptors (PRRs) including C-type lectin-like receptors (CLRs), Toll-like receptors (TLRs) and nucleotide oligomerisation domain (NOD)-like receptors (NLRs). IL-12 family members, IL-12p70 and IL-23, are important for host immunity against *Candida* spp. Herein we show that IL-27, another IL-12 family member, is produced by myeloid cells in response to select *Candida* spp.

Results: We demonstrate a novel mechanism for *C. parapsilosis*-mediated induction of IL-27 in a TLR7-, MyD88- and NOD2-dependent manner. Our data revealed that IFN-β is induced by *C. parapsilosis*, which in turn signals through the interferon-α/β receptor (IFNAR) and STAT1/2 to induce IL-27. Moreover, IL-27R (WSX-1) deficient mice systemically infected with *C. parapsilosis* displayed enhanced pathogen clearance compared to WT mice. This was associated with increased levels of pro-inflammatory cytokines in the serum and increased IFN-γ and IL-17 responses in the spleens of IL-27R deficient mice.

Conclusion: Thus our data define a novel link between *C. parapsilosis*, TLR7, NOD2, IFN-β and IL-27 and we have identified an important role for IL-27 in the immune response against *C. parapsilosis*. Overall these findings demonstrate an important mechanism for the suppression of protective immune responses during infection with *C. parapsilosis*, which has potential relevance for infections with other fungal pathogens.

In-host adaptation of *Aspergillus fumigatus*: a phenotypic and genotypic analysis

Eloise, Ballard[1], Willem J.G., Melchers[2], Alistair J.P., Brown[1], Paul E., Verweij[2], Adilia, Warris[1].

1. Aberdeen Fungal Group, MRC Centre for Medical Mycology, Institute of Medical Sciences, University of Aberdeen, UK 2. Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands

Background. In order to survive and thrive, *A. fumigatus* must adapt to specific niche environments. Examples of in-host adaptation include the development of azole resistance and modifications that enable persistent colonisation. The aim of this study is to gain insight into the types of genetic and physiological adaptation of *A. fumigatus* that occurs in patients. **Methods.** Central to this study are 13 *A. fumigatus* strains isolated from a single chronic granulomatous disease patient suffering from recurrent invasive aspergillosis. The strains were isolated over a period of 2 years. These strains were subjected to detailed phenotypic and genotypic analysis including whole genome sequencing. **Results.** All strains had identical microsatellite genotypes and were considered isogenic. The first 2 strains to be isolated were azole susceptible, whereas later isolates were itraconazole and posaconazole resistant (Table 1). Growth assays in the presence and absence of various antifungal stressors highlighted minor changes in growth rate and stress resistance; with the exception of isolate V157-62 which possessed a significant growth defect. Interestingly, later isolates produced significantly fewer conidia in comparison to earlier isolates. In specific resistant isolates conidiation was restored in the presence of itraconazole. Whole genome comparisons identified 248 non-synonymous single nucleotide polymorphisms thought to have developed in-host. **Conclusions.** These findings give insight into the vast genetic and physiological changes that occur in *A. fumigatus* during adaptation to the human host undergoing azole therapy.

Table 1. Minimum inhibitory concentrations of collection of isolates.

Temporal order of isolation	Strain	Minimum inhibitory concentration (mg/L)		
		Itraconazole	Voriconazole	Posaconazole
1	V130-15	1	1	0.25
2	V130-14	1	1	0.25
2	V130-18	4	4	0.5
2	V130-54	>16	1	0.125
3	V157-39	>16	1	>16
3	V157-40	>16	1	>16
3	V157-47	>16	2	>16
3	V157-48	>16	2	>16
3	V157-62	>16	8	>16

4	V157-59	>16	4	>16
4	V157-60	>16	4	>16
4	V157-61	>16	4	>16
5	V157-80	>16	1	>16

MICs exceeding the EUCAST clinical resistance breakpoint were considered resistant; itraconazole >2 mg/L, voriconazole >2 mg/L and posaconazole >0.25 mg/L

Selective transport between heterogeneous hyphal compartments via the plasma membrane lining septal walls of *Aspergillus niger*

Robert-Jan, Bleichrodt[1,2], Arman, Vinck[1], Nick, D. Read[2], & Han A.B. Wösten[1]

[1]Microbiology and Kluyver Center for Genomics of Industrial Fermentation, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[2]Manchester Fungal Infection Group, Institute of Inflammation and Repair, University of Manchester, CTF Building, 46 Grafton Street, Manchester M13 9NT, UK

Hyphae of ascomycetes are compartmentalized by septa. The central pore in these septa allows for cytoplasmic streaming. However, many of these pores are closed by Woronin bodies in *Aspergillus*, which prevents cytoplasmic mixing and thus maintains hyphal heterogeneity. Here, glucose uptake and transport was studied in *Aspergillus niger*. Glucose uptake was higher in the hyphal population with high transcriptional activity when compared to the population with low transcriptional activity. Glucose was transported from the colony center to the periphery, but not vice versa. This unidirectional flow was similar in the wild-type and the Δ*hexA* strain that does not form Woronin bodies. This indicated that septal plugging by Woronin bodies does not impact long distance glucose transport. Indeed, the glucose analogue 2-NBDG (2-(N-[7-nitrobenz-2-oxa-1,3-diazol-4-yl]amino)-2-deoxyglucose) translocated to neighboring hyphal compartments despite Woronin body mediated plugging of the septum that separated these compartments. Notably, 2-NBDG accumulated in septal cross walls, indicating that intercompartmental glucose transport is mediated by transporters that reside in the plasma membrane lining the septal cross-wall. The presence of such transporters would thus enable selective transport between heterogeneous compartments.

Rapid and robust identification of the agents of black-grain mycetoma by MALDI-ToF mass spectrometry

Mark Fraser, Andrew M. Borman, and Elizabeth M. Johnson

Public Health England UK National Mycology Reference Laboratory, Myrtle Road, Bristol BS2 8EL; email: Andy.Borman@uhBristol.nhs.uk

Eumycetoma is a debilitating, chronic, fungal infection endemic in India, Indonesia and parts of Africa and South and central America. It remains a neglected tropical disease in need of international recognition. Infections follow traumatic implantation of saprophytic fungi and frequently require radical surgery or amputation in the absence of appropriate treatment. Several fungal species can cause black-grain mycetomas, including *Madurella* spp. (*Sordariales*), *Falciformispora* spp., *Trematosphaeria grisea, Biatriospora mackinnonii, Pseudochaetosphaeronema larense, Medicopsis romeroi* and *Emarellia* spp. (all *Pleosporales*). Most of the common agents of black-grain eumycetoma produce dark grey, brown or black colonies, and dematiaceous hyphae which either remain sterile, or sporulate only very reluctantly, an absence of conidiogenesis that has historically hindered accurate species identification. Accurate identification of the agents of eumycetoma is essential to guide appropriate antifungal therapy. Since phenotypic identification of the causative fungi is often difficult, time-consuming molecular approaches are currently required and we, and many others, have previously reported that accurate species-level identification could be achieved by PCR amplification and sequencing of regions of the rDNA gene cassette. Here, we have subjected 59 isolates encompassing 10 different species of eumycetoma agents to MALDI-ToF analyses. After appropriate reference spectra were created and added to commercial databases, MALDI-ToF mass spectrometry accurately and rapidly identified all test isolates, with 100%, 92% and 69% of isolates giving Mean LogScores >1.8, >1.9 and >2.0, respectively. Moreover, intraspecific Mean LogScores were consistently, significantly higher than interspecific scores, demonstrating that MALDI-ToF allows rapid and robust identification of these recalcitrant organisms.

Rapid identification of clinically relevant members of the genus *Exophiala* by MALDI-ToF mass spectrometry and description of two novel species: *Exophiala campbellii* and *Exophiala lavatrina*

Andrew M. Borman[1], Mark Fraser[1], Adrien Szekely[1], Daniel E. Larcombe[2] and Elizabeth M. Johnson[1]

[1]*Public Health England UK National Mycology Reference Laboratory, Myrtle Road, Bristol BS2 8EL; email: Andy.Borman@uhBristol.nhs.uk and* [2]*School of Biological Sciences, University of Bristol, Bristol, UK*

Exophiala is a ubiquitous pleomorphic genus comprising at least forty species, many of which have been associated with superficial, visceral or systemic infections in humans, other mammals or cold-blooded animals. In this study we investigated the potential of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) for the identification of *Exophiala* species. A total of 89 isolates (including 50 human and 4 animal clinical isolates) stored in the National Collection of Pathogenic Fungi were identified by PCR amplification and sequencing of the internal transcribed spacer region 1. Eighty-three of the isolates corresponded to sixteen known species within *Exophiala/Rhinocladiella*. The remaining six isolates are shown by phylogenetic analyses based on four loci to represent two novel *Exophiala* species. Four isolates from domestic bathrooms which form a sister species with *E. lecanii-corni* are described here as *E. lavatrina* sp. nov. The remaining two isolates, both from subcutaneous infections, are distantly related to *E. oligosperma* and are described here as *E. campbellii* sp. nov. The triazoles and terbinafine exhibited low MICs against all *Exophiala* isolates *in vitro*. MALDI-ToF MS successfully distinguished all eighteen species and identified all isolates after appropriate reference spectra were created and added to commercial databases.

Intraspecific Mean LogScores ranged from 1.786 to 2.584, and were consistently, significantly higher than interspecific scores (1.193 to 1.624), with the exception of *E. lecanii-corni* and *E. lavatrina* for which there was considerable LogScore overlap. In summary, MALDI-ToF MS allows the rapid and accurate identification of a wide range of clinically relevant *Exophiala* species.

Structure-function analysis of Candidalysin, a recently identified fungal toxin

Nessim Kichik, Matthew Davy, David Moyes, Jonathan Richardson, Julian Naglik and Ernesto Cota

We recently identified Candidalysin as the first cytolytic peptide toxin in a human fungal pathogen. Candidalysin is an amphipathic 31-mer peptide capable of damaging epithelial cell (EC) membranes and inducing epithelial innate immunity. We aim to understand, at the atomic level, how Candidalysin causes membrane destabilisation and cell damage.

To study this toxin, we are employing a combination of biochemical and biophysical methods, such as cross-linking, circular dichroism and NMR. We observe that Candidalysin intercalates efficiently with detergent micelles that mimic the environment of the host cell membrane. We also show that once intercalated, Candidalysin oligomerises to form discrete structures that may be responsible for cell damage. To obtain a tridimensional structure of Candidalysin, our efforts are also focused on the production of recombinant, isotopically labelled samples for NMR spectroscopy. A molecular model of this oligomer will allow us to identify the amino acid residues critical for function and the molecular mechanism of this peptide toxin. Furthermore, this will provide essential information for rational design of anti-Candida vaccines, anti-proliferative agents and novel tools in biomedical research.

Does Differential Regulation of Hsp90 Cochaperones Contribute to Virulence in *Candida albicans*?

Debra DeLoach[1] & Stephanie Diezmann[1]

[1]*Department of Biology & Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, ddd22@bath.ac.uk*

Treatment options for systemic candidaemia, caused by the leading fungal pathogen *Candida albicans*, are limited by drug target paucity and emergence of antifungal drug resistances. Fungal virulence, morphogenesis, and drug resistance are regulated by the essential and highly conserved molecular chaperone heat shock protein 90 (Hsp90). While Hsp90 itself may be an unsuitable drug target, its less conserved cochaperones, which regulate Hsp90 ATP-dependent chaperoning of up to 10% of the proteome, may provide suitable venues for drug development if they regulate *C. albicans* virulence. When measuring protein levels of Hsp90 and five of its cochaperones, we noticed that cochaperones appear to be co-regulated during filamentous growth. Cdc37, Sba1, and Sti1, which block Hsp90 ATPase activity, have increased expression, while Cpr6 and Aha1, which support Hsp90 ATPase activity, have reduced levels relative to yeast planktonic growth. This suggests that Hsp90 cochaperone co-regulatory expression patterns may be linked to *C. albicans* virulence-associated hyphal morphology, though further studies are needed in other virulence-associated conditions. Our findings contribute to understanding the virulence potential and regulatory expression of Hsp90 cochaperones and thus will yield insights into fungal virulence strategies to further the development of Hsp90 cochaperones as future drug targets.

Pseudomonas aeruginosa can kill *Candida albicans* hyphae by mechanisms that require a functional Type 3 Secretion System

Emily Dixon[1] & Rebecca Hall

[1]Institute of Microbiology & Infection and the School of Biosciences, University of Birmingham, Edgbaston, UK efd018@bham.ac.uk

The opportunistic fungal pathogen *Candida albicans* is frequently co-isolated with *Pseudomonas aeruginosa* from the lungs of Cystic fibrosis patients, burn wounds and biofilms on indwelling medical devices. These opportunistic pathogens undergo a complicated series of interactions including quorum sensing dependent inhibition of virulence factor expression and direct cell-cell interactions resulting in hyphal death. Whilst secreted phenazines, including pyocyanin, have been shown to induce fungal death, the role of contact dependent killing mechanisms has not been investigated. *P. aeruginosa* expresses two contact dependent secretion systems (Type 3 and Type 6). The Type 3 Secretion System (T3SS) is a key virulence factor of *P. aeruginosa,* enabling the injection of cytotoxic effector molecules into mammalian and plant host cells. The T6SS can also inject effector molecules into eukaryotic hosts, but is also known to be involved in inter-bacterial competition. Therefore, we investigated the role of these contact dependent secretion systems in the binding and killing of fungal hyphae. Here we show that *P. aeruginosa* mutants defective in either the T3SS or T6SS still bound *C. albicans* hyphae, but only mutants defective in the T3SS showed a reduced ability to kill fungal hyphae during the initial phase of this interaction.

Further work will go on to determine the mechanism of this effect, including characterising the role of known effector proteins. Understanding the dynamics of the initial formation of complex polymicrobial communities will undoubtedly help to prevent their establishment.

Linking mitochondrial function to cell wall organisation and virulence in *Candida albicans*

Lucian Duvenage[1], Carol Munro[2] and Campbell Gourlay[1]

1. School of Biosciences, University of Kent, Canterbury, U.K.
2. Institute of Medical Sciences, University of Aberdeen, Aberdeen, U.K.

Candida albicans, one of the leading human fungal pathogens, relies mainly upon oxidative phosphorylation to support the metabolic requirements of morphogenesis and proliferation. Although connections have been suggested it is not well understood how the state of mitochondrial respiration influences virulence factors such as the composition of the cell wall, morphogenesis and host cell interaction. The effects of electron transport inhibition were tested on cell wall organisation, hyphal transition and macrophage interaction. Wild-type cells pre-treated with a combination of the nitric oxide donor sodium nitroprusside (SNP) and the alternative oxidase inhibitor (SHAM), led to cell wall re-arrangements. Elevated levels of exposed chitin and beta-glucans were observed compared to untreated cells. These same effects were observed in respiration-deficient mutant cells (*ndh51*Δ). The pre-treated cells also exhibited enhanced filamentation and more rapid uptake by murine macrophages. However, this pre-treatment of cells did not lead to any significant difference in survival as compared to the wild-type, in a zebrafish model of systemic candidiasis. Deletion of both alternative oxidases (Δ*AOX)* in *C. albicans* caused a delay in filamentation under hyphal-inducing conditions. Although the Δ*AOX* mutant was still virulent, zebrafish injected with this mutant survived longer, as compared to wild-type. These data suggest that inhibition of certain respiratory pathways can lead to cell wall reorganisation and changes in hyphal growth, which can have consequences for interaction with the immune system.

The fungus *Mucor circinelloides* induces platelet aggregation through integrin αIIbβ3 and FcγRIIa

Harlene Ghuman[1], Alicia Shepherd-Roberts[1], Stephanie Watson[2], Steve P Watson[2], Kerstin Voelz[1]

[1] School of Biosciences and Institute of Microbiology and Infection, College of Life and Environmental Sciences, University of Birmingham, Birmingham B15 2TT UK; [2] Institute of Cardiovascular Science, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham. B15 2TT

INTRODUCTION: The hallmarks of the invasive fungal infection mucormycosis are considered to be angioinvasion, thrombosis and tissue necrosis, with thrombosis in particular indicating a key role of platelets. However, there is currently little information on the interaction between platelets and mucormycetes under physiological conditions and the molecular signalling pathways underlying this interaction. The aim of this study was to determine the signalling pathway facilitating aggregation in response to M. circinelloides.

METHODS: Clinical isolate *Mucor circinelloides* NRRL3631 was used in this study. *M. circinelloides*-induced platelet aggregation was investigated in healthy, human whole blood via multiplate-aggregometry. Platelet aggregation and inhibition studies were investigated in PRP thereafter, over 30 min using light-transmission aggregometry. The developmental stages of spores were visualized via DIC light microscopy and analyzed using ImageJ.

RESULTS: *M. circinelloides* induces significant platelet aggregation in whole blood, and in a concentration-dependent manner in platelet-rich plasma. Aggregation potential is dependent on the spore's developmental stage, with strongest platelet aggregation by spores at 3 hours germination at the mid-point of maximal swelling, and weakest at 48 hours germination when hyphae are prevalent. Platelet aggregation in response to *M. circinelloides* is inhibited by inhibitors eptifibatide, mAb IV.3, Dasatanib and PRT-060318.

CONCLUSION: These results show that *M. circinelloides*-induced platelet aggregation is supported by platelet integrin αIIbβ3 and IgG receptor FcγRIIa, activating Src and Syk tyrosine kinase signalling. Furthermore, it is shown that platelet aggregation in response to *M. circinelloides* is dependent on spore developmental stage.

This work was supported by the British Heart Foundation (Ref: CH/03/003) and the Wellcome ISSF (Ref: 175ISSFPP)

Inhibition of macrophage functions by a secreted compound from mucorales

Herbert Itabangi[1], Ignacio Insua Lopez[2], Bradley Pollard[1], Joao Correria[1,] Paula Seoane[1], Jason King [4], Gordon Brown[3], Carol Munro[3], Francisco Fernandez Trillo[2], and Kerstin Voelz[1]

[1]Institute of microbiology and infection, School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; [2]School of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; [3]Aberdeen Fungal Group, School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Foresterhill Aberdeen, AB25 2ZD, UK; [4]Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK.

Mucormycosis is a life-threatening mold infection with overall mortality rates of 50%, yet up to 100% in patients with disseminated disease, prolonged neutropenia, and brain involvement. The disease is caused by a group of filamentous fungi, the mucormycetes, in patients with immunosuppression due to diabetic ketoacidosis (DKA), iron overload, severe trauma, neutropenia, corticosteroid treatment, or organ transplantation. These

predisposing conditions are linked to defects in key aspects of the innate immunity, particularly defects in phagocytic effector functions by macrophages and neutrophils. Yet, we currently have a very limited understanding of the molecular interaction between mucormycete spores and phagocytes. Here, we report a compound secreted by mucormycete spores that inhibits macrophage functions including phagocytic uptake, phagosome acidification and pathogen killing. We characterize this compound in a series of physical and biochemical interventions coupled with high performance liquid chromatography.

Fungal and bacterial secreted factors regulate mucormycete germination

Courtney Kousser[1], Kerstin Voelz[1], & Rebecca A Hall[1]

[1]*Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, UK*

Within the human body, microorganisms reside as part of a complex and varied ecosystem, where they rarely exist in isolation. Therefore, bacteria and fungi have co-evolved to develop elaborate and intricate relationships, utilising both physical and chemical communication mechanisms. Mucormycetes are spore-forming fungi belonging to the order Mucorales and are the causative agent of potentially fatal mucormycosis in immunocompromised individuals. Current therapeutic interventions involving surgical removal of infected tissues and antifungal treatment are often ineffective, with almost 100% mortality occurring in disseminated mucormycosis. Key to the pathogenesis of mucormycetes is the ability to swell and germinate leading to penetration of the surrounding tissues, angioinvasion, vessel thrombosis, and tissue necrosis. These spores are found ubiquitously and most likely encounter a myriad of bacterial and fungal species. For example, mucormycetes have been co-isolated with other trauma- and burn- associated pathogens, such as *Candida albicans* and *Pseudomonas aeruginosa* as well as pulmonary- associated microorganisms such as *Klebsiella pneumoniae*. This study demonstrates that exposure of *Rhizopus microsporus* spores to other pathogenic microbes can inhibit germination. Therefore, it is possible that a predisposing bacterial or fungal infection is able to regulate germination and influence overall fungal pathogenicity through secreted factors.

From Bug to Drug: In-house production of bespoke antifungal peptide aptamers

Bethany McCann[1], Christopher Grice[1], Darren Thomson[1], Margherita Bertuzzi[1], & Elaine Bignell[1]

[1]*Faculty of Biology, Medicine and Health, University of Manchester, Manchester Fungal Infection Group, 46 Grafton Street, Manchester, M13 9PT, United Kingdom, email: bethany.mccann@postgrad.manchester.ac.uk*

Of around 250 *Aspergillus* species, *Aspergillus fumigatus* is the most pathogenic to humans causing invasive fungal infections, and killing upwards of 200,000 people annually. However the number of available antifungal drugs is limited and occurrence of antifungal resistance is increasing.

Peptide aptamers (PAs) can be defined as short; 8-20 amino acids in length, combinatorial peptide fragments doubly constrained within inert protein scaffolds which exhibit functionality much like antibodies. High specificity and affinity for target proteins permits PAs the ability to modulate functionality of targets. Such ligand based

perturbation of function paves the way for the elucidation of novel drug targets and the design of potential inhibitory agents against virulence-causing mechanisms.

A. fumigatus mutants lacking the pH-dependent transcription factor PacC, or an upstream pH-responsive receptor protein PalH, display highly attenuated virulence resulting in non-invasive phenotypes. Inhibition of PalH mediated pH signalling is therefore an attractive novel antifungal strategy.

In an initial screen for selection of anti-PalH PAs, we used a yeast membrane two hybrid approach expressing the PalH receptor as bait, and a library of thioredoxin constrained peptide aptamers as preys. The approach successfully identified PalH-binding PAs, however in all instances expression in *A. fumigatus* delivered gain of pH function phenotypes. We describe the development of two critical new tools, of PAs causing loss of pH signalling, using direct selection in *A. fumigatus*: a randomised library of Peptide Aptamers (PAs) expressed, under regulatable control, in *A. fumigatus,* additionally a split-fluorescence approach for direct detection of protein-protein interactions, and their subsequent inhibition.

Longitudinal small-scale study of biofilm composition in relation to patient factors amongst voice prosthesis users

Ijeoma Okoliegbe & Alexandra C. Brand

MRC centre for Medical Mycology, Aberdeen Fungal Group, University of Aberdeen, UK
iokoliegbe@abdn.ac.uk

Voice prostheses (VPs) in total laryngectomy patients are affected by mixed-species biofilm formation that causes valve occlusion, device failure and frequent device replacement. We aimed to determine whether the presence of specific microbes, or combinations of them, is a marker for premature device failure and to explore the extent to which patient factors e.g. use of common therapeutics, influence biofilm composition. In addition we sought to establish the source of the biofouling. A 12-month longitudinal study was conducted within a cohort of 14 patients, to determine variation in the composition of VP biofilms and microbial carriage from accompanying oral rinses (OR). Associations with patient factors within and between participants were also sought. Microbial isolation from 57 devices showed that each participant exhibited an individual microbial profile and the VPs of all but one participant carried fungi, primarily *Candida* spp. Device replacement frequency was not linked to the carriage of specific organisms or to denture use but appeared to be reduced in participants using antacids. Multi-locus sequence typing of *C. albicans* and *C. glabrata* isolated from VPs and their accompanying OR sample suggested that the oral flora seeds VP biofilm formation, although *C. krusei* was commonly isolated only from the VP. The cohorts of microbes obtained will be used for comparative studies of drug resistance, biomass production and inter-species dependency.

The Candidalysins are a family of fungal toxins

Jonathan P. Richardson[1], Shir-Lynn Tan[1], Giulia Carrano[1], David L. Moyes[2] and Julian R. Naglik[1].

[1]Division of Mucosal and Salivary Biology, The Dental Institute, King's College London, London, UK; [2]Centre for Host-Microbiome Interactions, Mucosal & Salivary Biology Division, The Dental Institute, King's College London, UK. Email: jonathan.richardson@kcl.ac.uk

Candida albicans infections cause mucosal and life threatening systemic ailments that contribute to high morbidity and mortality worldwide. During mucosal infection, *C. albicans* secretes Candidalysin, a 31 amino acid toxin derived from its parent protein, Ece1p. Epithelial cells respond to Candidalysin through a bi-phasic p38/c-Fos response resulting in a strong inflammatory response at mucosal surfaces. Alignment of Ece1p amino acid sequences from different *Candida* species enabled the identification of additional putative Candidalysin toxins in *C. dubliniensis*, *C. tropicalis*, *C. albicans* 529L and *C. maltosa*. These peptide sequences were synthesised and evaluated for their ability to cause damage, activate mucosal inflammatory responses and the p38/c-Fos response pathway in oral (TR146) and vaginal (A431) epithelial cells. All putative toxins caused epithelial damage as evidenced by lactate dehydrogenase activity and induced epithelial signalling and cytokine secretion. Notably, the putative toxins of *C. tropicalis* and *C. dubliniensis* exhibited greater potency *in vitro* when compared with the Candidalysin of *C. albicans*. Our data suggest conservation of function despite differences in amino acid sequence and identify the Candidalysins as a family of functionally related fungal toxins.

Characterisation of *Rhizopus oryzae* spore germination, using RNA sequencing, live cell imaging and flow cytometry

Poppy Sephton-Clark, Kerstin Voelz

School of Biosciences, University of Birmingham, Edgbaston, UK; pxs260@bham.ac.uk

Rhizopus delemar, a fungus belonging to the mucorales order, is saprophytic and found ubiquitously. Mucorales species are responsible for causing Mucormycosis, an emerging infection that has proven difficult to treat, presenting with unacceptably high mortality rates in immunocompromised individuals. Disease depends upon spore germination and hyphal growth leading to angioinvasion and subsequent vessel thrombosis and necrosis in the host. However, we currently have a very limited understanding on the genetic network underpinning the regulation of mucormycete spore germination.

We have determined the phenotypic changes that occur during germination of *Rhizopus delemar* RA99-880 spores by defining spore swelling, hyphal growth patterns and changes in cell wall composition by live-cell imaging and flow cytometry. We combined our phenotypic analysis with a high-resolution transcriptomics time course. We will present our phenotypic data in correlation with RNA-Seq data identifying differential gene expression during the germinating of *Rhizopus oryzae* spores. This is the first detailed study of the genetic regulation of mucormycete spore germination.

Adaptation to acidic environments induces unmasking of beta-glucan in *Candida albicans*

Sarah Sherrington & Rebecca Hall

Institute of Microbiology and Infection, University of Birmingham, Edgbaston, Birmingham, UK; s.sherrington@bham.ac.uk

The fungal pathogen *Candida albicans* has the ability to thrive within a variety of host niches. These different body sites vary dramatically in environmental factors including pH, CO_2 levels and the presence of quorum sensing molecules. Although these environmental parameters have been extensively investigated for the ability to regulate morphogenesis and white-opaque switching of *C. albicans*, their role in regulating cell wall biosynthesis is largely unknown. Here we investigate how adaptation to acidic environments affects the architecture and biosynthesis of the fungal cell wall. Growth in acidic environments (pH4) resulted in significant exposure of the underlying beta-glucan, which was associated with enhanced innate immune recognition mediated via Dectin-1. Investigation into the conventional signalling pathways that regulate adaptation to environmental pH and cell wall stress, suggested that this unmasking of beta-glucan is regulated via a non-canonical signalling mechanism. To elucidate key genes involved in this phenotype we have screened a library of *C. albicans* mutants for their ability to undergo pH dependent beta-glucan unmasking. This screen identified several genes that may function in the unmasking of beta-glucan in response to environmental stimuli, and also identified genes that function in general cell wall biogenesis. We are now beginning to uncover the molecular mechanisms driving β-glucan unmasking, in the hope to better understand the dynamic nature of the *Candida* cell wall within different host environments.

Initial investigation into the host pathogen-interactions of *Prototheca*, an understudied but fascinating pathogenic alga

Leanne Stones, Alan McNally & Robin May

IMI, School of Biosciences, University of Birmingham, Birmingham, UK, *l.stones@bham.ac.uk*

Prototheca are a genera of pathogenic, achlorophyllous algae found ubiquitously in nature associated with the slime-flux of trees, plant surfaces and water. They are a common and costly cause of bovine mastitis in the dairy industry but also cause the rare human condition protothecosis, which is often the result of traumatic inoculation. Disease is mainly restricted to the subcutaneous tissue in immunocompetent patients but olecranon bursitis is another distinctive presentation. In immunocompromised patients *Prototheca* can result in disseminated disease typically affecting the internal organs of the abdomen. Although first identified as a cause of human disease in 1964, little is known about the pathogenicity of this unicellular, 'yeast-like' alga and no genome sequences have been deposited in genbank to aid their study.

Here we have begun to look at the two most common species associated with human disease; *Prototheca wickerhamii* and *Prototheca zopfii* (genotypes 1 and 2). Our preliminary work has shown that a subset of cells from three species are able to release their endospores whilst contained within a phagocytic vacuole of a human monocyte derived macrophage (MDM); this appears to result in the destruction of the MDM and to the release of viable daughter cells. We have also been able to sequence the genomes of the three species of *Prototheca*, which range from 25-29Mb in size. Through further cellular studies and analysis of the genomic data we hope to determine how this unusual pathogen interacts with the human host.

A new iPSC model for Cryptococcal infection

Leanne M. Taylor-Smith & Robin C. May

Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.

Cryptococcus neoformans is a lethal fungal pathogen of immuno-compromised individuals, initially infecting the human lung before disseminating to and infiltrating the host central nervous system, leading to cryptococcal meningoencephalitis. Macrophages of the host are known to have a vital role in the innate immune response. However, it is well known that cryptococci are able to replicate within the macrophage phagosome and escape the phagocyte in a non-lytic manner termed vomocytosis. The molecular detail that regulates and allows this replication, escape and probable dissemination is key to developing new therapies. Therefore our group is interested in understanding how cryptococci manipulate phagocytes. Our most recent endeavors have focused on the use of human adult induced pluripotent stem cells (iPSCs) as a potential new model for cryptococcal infection and genetic manipulation of host cells. Induced adult stem cells have the advantage of being associated with fewer ethical issues compared to embryonic stem cells and yet are pluripotent and able to be differentiated into myriad cell types. With the help of collaborators, we have focused on new techniques to produce monocytes derived from iPSC mesoderm embryoid bodies. These cells are then further differentiated for 7 days to macrophages. Preliminary results so far suggest the iPSC-derived macrophages are capable of phagocytosis of cryptococcal cells, are able to harbor intracellularly replicating *Cryptococcus* and are permissive to the processes of vomocytosis and lateral transfer.

Effects of Mincle and Dectin-1 on Myeloid Cell Function

Aiysha Thompson[1] & Selinda J. Orr[1]

[1]Division of Infection & Immunity, Cardiff University School of Medicine, Cardiff, CF14 4XN.

Introduction: Invasive fungal infections, including candidiasis, account for ~1.5 million deaths per year worldwide. Candidiasis is the fourth most common healthcare-associated infection, killing more people per year than TB or malaria. Several strains of *Candida* cause candidiasis however >95% of *Candida* infections are caused by *C.albicans* (54%), *C.glabrata* (19%), *C.tropicalis* (11%) and *C.parapsilosis* (11%). Current anti-fungal therapies are often insufficient, as the mechanisms regulating anti-fungal immunity are not fully understood. Fungal infections cause a complex immune response involving complement receptors (CRs), C-type lectin-like receptor (CLRs), Toll-like receptors (TLRs) and others. A better understanding of anti-fungal immunity is required to develop novel immunotherapies.

Results: To examine the importance of CLRs in antifungal immunity, we have performed a comprehensive analysis of the role of Dectin-1 and Mincle in different myeloid cells in response to *C.albicans*. Expression levels of Dectin-1 and Mincle on bone marrow derived macrophages (BMDM) and bone marrow derived dendritic cells (BMDC) were measured. BMDM from WT, Dectin-1 deficient and Mincle deficient mice were cultured and cytokine production (TNF, IL-1b, IL-10, IL-12p40 and IL-6) in response to *C.albicans* was measured by ELISA. WT, Dectin-1 deficient and Mincle deficient mice were infected with a low and high dose of *C.albicans* and T cell responses (IFN-g and IL-17), fungal burden and survival were measured.

Conclusion: Dectin-1 is important for cytokine production, T cell responses and survival. The role for Mincle during *C.albicans* infections is more subtle, however we have observed small effects from Mincle in the different aspects of anti-fungal immunity.

Early phagocyte recruitment controls the opportunistic fungal pathogen *Mucor circinelloides* in nascent granulomas *in vivo*

Aleksandra Jasiulewicz[X] and Kerstin Voelz[X]

[X] *Institute of Microbiology & Infection, University of Birmingham, Edgbaston, Birmingham , B15 2TT, UK;* k.voelz@bham.ac.uk

Granulomatous structures have been described in histopathological examinations for a large spectrum of opportunistic fungi (e.g. mucormycosis, cryptococcosis, candidiasis, chromoblastomycosis). In the well-studied bacterial granuloma of tuberculosis, these highly structured complexes of immune cells contain, but do not eradicate, mycobacteria. Macrophages and neutrophils can control TB by slowing down bacterial growth during early infection, yet also function as vehicles for later tissue dissemination. Granuloma formation during TB may provide both a means to control infection but also a niche for bacteria to survive, waiting for an opportunity to break out and cause disease. However, so far, the role of granulomas during fungal infections has been overlooked.

We used the zebrafish larval model, *Danio rerio*, to determine the role of innate immune responses to mucormycete spores *in vivo*. Upon infection with spores, neutrophils and macrophages are rapidly recruited to the site of infection to form tight clusters around fungal spores. Single cell analysis revealed that the number of macrophages and neutrophils co-localising sharply increases 3 hours post infection indicating the emergence of a nascent granuloma. Although innate immune cells are readily recruited to the site of infection, fungal spores remain viable within these tight granulomatous structures over the course of the experimental observation. Following immunosuppression with dexamethasone, phagocyte recruitment and granuloma formation are significantly reduced whilst dissemination and larval mortality is increased.

Taken together, this data indicate that early, rapid recruitment of phagocytes to the site of infection controls this opportunistic fungal pathogen by retaining infectious spores in nascent granulomatous clusters.

Kindly Supported by:

GILEAD
Advancing Therapeutics.
Improving Lives.

basilea
PHARMACEUTICA

WELLCOME TRUST STRATEGIC AWARD
mmfi
MEDICAL MYCOLOGY & FUNGAL IMMUNOLOGY

MSD

Pfizer

ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection

BRUKER
Bruker Microbiology

olm diagnostics

IMI INSTITUTE OF MICROBIOLOGY AND INFECTION

NON SOLUS
ELSEVIER