

Host-fungal interactions: pathogenicity versus immunity

The Penridge Suite, 470 Bowes Road, London N11 1NL: 21 October 2011 09:00 - 17:00

Fungal pathogens cause a range of serious mucosal infections and life-threatening fungaemia in a variety of immunocompromised hosts. This EuroSciCon meeting on host-fungal interactions aims to be a premier forum in the UK for presentation of cutting-edge advances in relation to fungal pathogenicity and innate and adaptive immune mechanisms against pathogenic fungi. The meeting will help chart the course of future research and facilitate the urgent necessity to understand, treat, and prevent disease.

Meeting Chairs:

Dr Julian R. Naglik, King's College London, United Kingdom and
Professor Neil Gow, University of Aberdeen, UK

This event has CPD accreditation

- 9:00 – 9:45 **Registration**
- 9:45 – 10:00 **Introduction by the Chairs:** Dr Julian R. Naglik, King's College London, United Kingdom and Professor Neil Gow, University of Aberdeen, UK
- 10:00 – 10:30 **Host-fungal interactions: pathogenesis and importance of morphology**
Dr Bernhard Hube, Hans-Knoell-Institute, Germany
One of the most frequently discussed virulence attributes of the pathogenic fungus *Candida albicans* is the ability to change growth morphology from spherical yeast cells to elongated hyphae (dimorphism). Multiple studies have investigated and elucidated the network of regulators controlling the yeast-to-hypha transition, and the molecular and cellular events associated with dimorphism. However, the distinct pathogenic roles of the two morphological forms are less clear. In this talk, the biological properties of yeast and hyphae and their roles in interaction with the host will be discussed.
- 10:30 – 11:00 **Pattern recognition receptor and host fungal interactions**
Professor Gordon Brown, University of Aberdeen, Scotland
The innate ability to detect pathogens is essential for multicellular existence, and has been achieved through the evolution of germ-line encoded receptors which can recognise non-self structures, the so-called "pattern recognition receptors" (PRR). In this presentation we will discuss the role of selected PRRs in anti-fungal immunity.
- 11:00 – 11:05 **Speakers photo**
- 11:05 – 11:30 **Mid-morning break**
- 11:30 – 12:00 **Adaptive immunity to fungal pathogens: new insights**
Dr. Caetano Reis e Sousa, Cancer Research UK
- 12:00 – 12:30 **Macrophage-fungal interactions during cryptococcosis**
Dr Robin May, School of Biosciences, University of Birmingham, UK
Cryptococcus neoformans and *Cryptococcus gattii* are the causative agents of cryptococcosis, a fatal infection of the central nervous system. Upon entry into the lung, cryptococci are engulfed, but not destroyed, by phagocytic cells. Within this privileged location, they are able to replicate, traffic to distant organs and then escape using a novel mechanism that we have termed 'vomocytosis'. I will discuss our recent data on vomocytosis and how host macrophages attempt to block it. In addition, I will talk about macrophage interactions in hypervirulent outbreaks such as that which is currently occurring in Canada and the USA.
- 12:30 – 13:30 **Lunch**
- 13:30 – 14:30 **Question and Answer Session**
Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

- 14:30 - 15:00 **Als adhesins - Structural basis for the broad specificity to host-cell ligands by *Candida albicans***
Dr Ernesto Cota, Imperial College, London .
 Als (agglutinin-like sequence) glycoproteins have been associated with binding of host-cell surface proteins and small peptides of random sequence, the formation of biofilms and amyloid fibers. High-resolution structures of N-terminal Als domains show that ligand recognition relies on a motif capable of binding flexible C termini of peptides in extended conformation. These data establish NT-Als adhesins as a separate family of peptide-binding proteins and an unexpected adhesion system for primary, widespread protein–protein interactions at the *Candida*/host-cell interface. The new structural information also provides potential templates for the design of novel antifungals.
- 15:00 - 15:30 **Afternoon Tea/Coffee**
- 15:30 – 16:00 **Live cell imaging of *Candida*-Macrophage interactions**
Dr Lars Erwig, University of Aberdeen, UK
 We have employed live cell video microscopy to conduct a comprehensive analysis of *Candida*-Macrophage interactions in vitro. This includes detailed analysis of macrophage migration towards fungal pathogens, engulfment after cell-cell contact and studies of phagosome maturation and macrophage killing.
- 16:00– 16:30 **Sensing and adapting to the mammalian host: A global view of the *A. fumigatus* infectious transcriptome**
Dr Elaine Bignell, Imperial College London, UK
 In cases of aspergillosis the major underlying determinant of infectious outcome is host immunity. To understand how *A. fumigatus* grows and develops in the host, our group has intensively studied the early phases of infection by looking at the transcriptome. Our research seeks to identify factors which drive fungal pathogenicity in the whole animal host with a view to informing clinical progress in the areas of therapeutics and diagnostics.
- 16:30 - 17:00 **Chairman's summing up**

About the chairs

Dr Julian Naglik currently directs the Epithelial Research Programme within the Clinical & Diagnostic Sciences Group at King's College London Dental Institute. He graduated in microbiology and Immunology (BSc, University of East London), obtained his Ph.D. in 2001 (King's College London) and was appointed Lecturer in 2006. He is course coordinator of Host Defence and Resistance to Infection (Immunology) for BDS (Bachelor of Dental Surgery) students. Active research projects relate to the molecular analysis of host/pathogen interactions, *Candida* and HIV pathogenesis, and host immunity at mucosal surfaces. The ultimate goals are to understand the disease process and to translate research discoveries into clinical practice.

Professor Neil Gow graduated with a B.Sc. from Edinburgh University, a Ph.D. from Aberdeen University and was a research fellow in Denver, before returning to Aberdeen in 1984. He is a founding member of the Aberdeen Fungal Group that constitutes one of the largest academic centers for medical mycology. He is President-Elect of ISHAM, former British Mycological Society President, and editor in Chief of the journal *Fungal Genetics and Biology*. His research is focused on: (i) the molecular genetics of cell wall biosynthesis in pathogenic fungi - in particular the genetics of glycosylation and the fungus-host interaction in relation to immune recognition and function, (ii) chitin synthesis and the response to antifungal agents; (iii) directional growth responses of fungal cells; (iv) the virulence properties of medically important fungal species; (v) the evolution, genome biology and genotyping of *Candida* species. He has published over 200 research papers and reviews in these areas.

Media Sponsors



Natural Standard **pharmaphorum**[™]
 bringing healthcare together

About the speakers

Robin May is a Lister Prize Fellow and Senior Lecturer in Infectious Disease at the University of Birmingham. Prior to joining the University of Birmingham in 2005, he was a Human Frontier Science Program fellow at the Hubrecht Laboratory, The Netherlands, where he worked on the mechanism of RNA interference in *C. elegans*. Previously, he studied plant sciences at the University of Oxford before carrying out his PhD research with Laura Machesky, on the role of the actin cytoskeleton in phagocytosis and intracellular infections.

Dr. Lars- Peter Erwig has a longstanding interest in the role of macrophages in the progression and healing of inflammation. His initial work has focussed on the signals that activate macrophages in vitro and glomerulonephritis and he has developed the concept of macrophage programming to explain how macrophages function within inflamed or otherwise damaged tissue where they are exposed to complex environments. His research group now focuses on the consequences of apoptotic cell uptake for phagocyte function and in particular on how the digestion of ingested cells or pathogens is controlled within macrophage phagosomes

Elaine Bignell, graduated from the University of East Anglia in 1991 with a BSc (Hons) degree in biochemistry. Her Phd and postdoctorate studies (conducted at Imperial College London) focused upon pH sensing and adaptation in *Aspergillus* species with particular eference to infection. Having secured a fast tracked position to Lecturer at Imperial College London in 2006 she was awarded an MRC New Investogator Award and has since established a research group which studies the role of environmental sensing during initiation of fungal infection, with a view to informing future therapeutic strategy.

Gordon Brown completed a Ph.D. in microbiology at the University of Cape Town, South Africa. He was a Wellcome Trust travelling postdoctoral fellow at the University of Oxford, UK, then a Wellcome Trust Senior Fellow at the University of Cape Town, South Africa, and is now a Professor of Immunology at the University of Aberdeen. His primary research interests are macrophage receptors and their role in immunity and homeostasis.

Bernhard Hube is head of the Department of Microbial Pathogenicity Mechanisms at the Hans Knoell Institute (HKI) of the Leibniz Society in Jena, Germany. His research is focused on the pathogenesis of mycoses caused by yeasts such as *Candida albicans* or *C. glabrata*. He is principal investigator or vice speaker of international graduate schools (ILRS, JSMC) and the Center for Sepsis Control and Care (CSCC), member of several advisory boards, vice president of the International Society for Human and Animal Mycology (ISHAM) and has been awarded with several scientific awards and a fellowship in the American Academy of Microbiology (AAM).

Dr Ernesto Cota is currently a Lecturer at Imperial College London. He graduated with a BSc in Biology, an MSc in Biotechnology from the National University of Mexico (UNAM) and a PhD in Protein Chemistry in the University of Cambridge. Since 2000, he has been studying mechanisms of microbial adhesion using structural techniques, in particular using models from pathogenic *E. coli*. Employing the same approaches, he has recently focused on adhesion mechanisms in *C. albicans*, based on the idea that adhesion molecules are primary targets for the design of new antifungals, as they constitute the first line of interaction with host cells and tissues.

Connect with us on

Nature network - <http://network.nature.com/groups/euroscicon/>

LINKEDIN- <http://www.linkedin.com/groups?gid=1939569>

Facebook- <http://www.facebook.com/group.php?gid=70847076549>

Twitter - <http://twitter.com/Euroscicon/>

Dont forget to sign up to Euroscicons' e-newsletter at www.euroscicon.com/signup.htm to keep up to date with European Life Science news and events and to be notified of the follow up to this event

Keywords: C-type lectins, antifungal immunity, phagocytes, Infection, *Aspergillus fumigatus*, transcriptomics, virulence, fungi, *Candida albicans*, dimorphism, adhesion, invasion, damage, Tissue tropism/ *Candida* adhesion/ Als adhesin/ Structure/ Biofilms

Registration Web Site: www.regonline.co.uk/fungal2011

STAGE-SPECIFIC RAB GTPASE FUNCTION IN PHAGOSOMES CONTAINING *CANDIDA ALBICANS*: TOOLS TO RESOLVE THE MOLECULAR MECHANISMS OF PATHOGEN-PHAGOCYTE INTERPLAY

Bain, J., Okai, B., Lewis, L., Pratt, L., McKenzie, C., Erwig, L-P.

Immunology and Infection, Division of Applied Medicine, School of Medicine and Dentistry,
University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD.

Phagosomes acquire degradative properties as they mature. Maturation is regulated by Rab GTPases; the role of Rab5 and Rab7 in this process is well characterised. Several pathogens have evolved mechanisms to subvert phagosome maturation by manipulating Rab function. The clinically important pathogenic fungus, *Candida albicans*, is able to escape the phagosome and subsequently, the macrophage, by producing hyphal filaments. Combining a *C. albicans* phagocytosis model with sophisticated live video microscopy, we studied Rab GTPase activity in maturing phagosomes using siRNA and GFP-/RFP-tagged variants of native and mutant Rabs. In addition to Rab5 and 7, we are studying Rabs with poorly defined phagosomal function (Rab2, 9, 10, 11, 14, 18, 22a, 23 and 35). As expected, siRNA knockdown of Rab7 in macrophages blocked phagosome maturation as demonstrated by reduced acidification of phagosomes containing yeast. Interestingly, Rab14, which is known to actively block phagosome maturation upon *Mycobacterium* phagocytosis, has the inverse effect on *Candida* phagosomes. Rab14 siRNA knockdown did not affect uptake of *C. albicans* but was associated with a 5-fold increase in macrophage lysis by hyphae. Confocal and live microscopy demonstrates localization of GFP-Rab14 to phagosomes that contained *C. albicans*. These results suggest that either Rab14 promotes phagosome maturation following phagocytosis of *C. albicans*, or that Rab14 participates in phagosomal membrane acquisition to accommodate the growing hypha. This approach provides mechanistic insight into the molecular processes driving phagosome maturation at the pathogen-phagocyte interface and may identify novel targets for therapeutic intervention.

CANDIDA ALBICANS CHITIN: AN IMMUNE REGULATORY MOLECULE?

J. Wagener, F.J.Alvarez, R.Hall and N.A.R.Gow

Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD
Corresponding author: J.Wagener@abdn.ac.uk

Candida albicans is the major systemic fungal pathogen of humans. Normally be part of the intestinal and vaginal micro flora, the fungus causes superficial infections of mucosa and skin, predominantly in immunocompromised individuals. Besides this, *Candida* can cause life-threatening systemic infections, from which approximately 35 % of the patients will not recover. One of the foremost factors in interactions between *C. albicans* and host immune cells is the fungal cell wall. This structure plays important roles in antigenicity, drug resistance and modulation of the host immune response.

The cell wall of *C. albicans* is comprised of an inner layer of chitin, β -1,3- and β -1,6-linked glucans and an outer layer rich in proteins modified with *N*- and *O*-linked mannans. These highly glycosylated mannoproteins are involved in adhesion to host cells, and, along with β -glucans known to modulate host immune response. Chitin on the other side, a homopolymer of β -1,4-*N*-acetylglucosamine (GlcNAc) is a component of the cell walls of almost all fungi, abundant in humans and therefore of main interest as drug target. Despite this little is known about the role of fungal chitin as potential immune regulatory molecule, but crab shell derived chitin for example is known to act as anti-inflammatory regulator in infections and autoimmune diseases. Moreover, a possible receptor recognising chitin is not known yet. Therefore we are interested to investigate the role of fungal chitin in innate immune recognition. Previous studies showed that chitin blocks recognition of *C. albicans* yeast cells by cells of the host immune system (Mora-Montes H *et al.*, 2011) and besides this, pre-treatment with *C. albicans* chitin enhance the survival of *C. albicans* infected mice (Rementeria A *et al.*, 1997).

For our investigations chitin from *C. albicans* were isolated, purified and used to stimulate human peripheral blood mononuclear cells (hPBMCs) alone or together with well known agonists of different pattern recognition receptors (PRRs). Cytokine secretion were analysed and our data show, that *C. albicans* derived chitin alone only moderately induced pro-inflammatory cytokine secretion of TNFalpha, IL-6 and IL-1beta. On the other hand chitin significantly increased the secretion of the anti-inflammatory cytokine IL-10. Pro-inflammatory cytokine secretion can be enhanced by forcing chitin uptake through liposomal transfection and is abolished by endocytosis blocking. However, IL-10 secretion of hPBMCs is potentiated by co-stimulation with all tested PAMPs, except CpG ODN, stimulating endosomal TLR9. Overall our results underline the anti-inflammatory role of chitin inducing IL-10 secretion and moreover indicate three different chitin-receptor interaction sides, the cell surface, in endosomes and/or the cytoplasm.

MANNOSYLATION OF *CANDIDA ALBICANS* IS IMPORTANT FOR IMMUNE RECOGNITION

Rebecca A. Hall, Steve Bates, J. Wagener, F. J. Alvarez, Frank C. Odds, Alistair J. P. Brown and Neil A. R. Gow

School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD

The fungal cell wall is comprised of an inner skeleton of chitin, β -1,3 glucan and β -1,6 glucan and an outer layer of heavily glycosylated mannoproteins. These cell wall components act as pathogen associated molecular patterns (PAMPs) which are recognised by pathogen recognition receptors (PRRs) on cells from the innate immune system. Our laboratory has generated a series of mannosylation mutants, which express different *N*- and *O*-mannan structures, which have demonstrated the importance of mannosylation in fungal immune recognition. To further characterise the role of α -1,2 mannose containing epitopes in fungal pathogenicity, the α -1,2 mannosyltransferase family (*MNN2*) was deleted in *C. albicans*. This family of enzymes are required for the elaboration of side chain mannan on the α -1,6 mannose backbone of *N*-linked mannans. Six orthologues of the *Saccharomyces cerevisiae* *MNN2* gene were identified in the *C. albicans* genome. Deletion of single genes confirmed that *MNN2* and *MNN26* were both required for the incorporation of the acid liable phosphomannan into the *C. albicans* cell wall and cell wall integrity. Deletion of *MNN2* resulted in a 70% decrease in TNF α production from peripheral blood monocyctic cells (PBMCs), while deletion of *MNN21*, *MNN22*, and *MNN26* resulted in a 50% reduction in TNF α production, compared to the parental control strain, confirming that α -1,2 mannose is important for immune recognition. The secreted levels of IL-6 and IL-1 β were not altered by the depletion of α -1,2 mannose. To examine the functional redundancy between the family members, double, triple, quintuple and sextuple mutants were made according to a phylogenetic profile of the gene family. Deletion of multiple *MNN2* family members further decreased cell wall integrity, with the sextuple mutant displaying extreme sensitivity to cell wall perturbing agents like Calcoflour White, Congo Red and SDS. The multiple *MNN2* mutants displayed a similar immune recognition profile to the single *MNN2* mutant, suggesting that *MNN2* and *MNN26* might be responsible for the addition of the first α -1,2 mannose linkages to the α -1,6 mannose backbone, with the other family members extending the α -1,2 mannose branch.

ANALYSIS OF MACROPHAGE MIGRATION TOWARDS AND ENGLUFMENT OF *CANDIDA ALBICANS* USING SOPHISTICATED LIVE CELL VIDEO MICROSCOPY

L. E. Lewis, J. M. Bain, T. Lowes, C. Gillespie, N. A. R. Gow and L-P. Erwig

Division of Applied Medicine, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK

Candida albicans is an opportunistic fungal pathogen that can cause life-threatening systemic infections in immunocompromised hosts. Phagocytosis of *C. albicans* by cells of the innate immune system is an essential component of the immune response to infection. We show here for the first time a detailed minute by minute account of the specific effects of *C. albicans* viability, cell wall composition, morphogenesis and spatial orientation on macrophage migration and engulfment of bound *C. albicans*.

Analysis of macrophage paths towards *C. albicans* using sophisticated tracking software revealed that the speed of macrophage migration was dependent on the glycosylation status of the fungal cell wall, but not on *C. albicans* viability or morphogenic switching from yeast to hyphal forms.

Macrophages rapidly engulfed viable and UV-killed *C. albicans*, but the rate of engulfment was significantly slower for all yeast-locked morphogenetic and glycosylation mutants examined. Hyphal cells were engulfed at a slower rate than yeast cells, especially those with hyphae in excess of 20 μ m, but there was no correlation between hyphal length and the rate of engulfment below this threshold. We showed that spatial orientation of the hypha was another important determinant of the rate of engulfment.

This study reveals unique insight into the complex mechanisms that govern *C. albicans* phagocytosis by macrophages and could serve as a blueprint for the study of host interactions with other pathogens and dying cells.