MICROBIOLOGY

TIME: 15.25 – 16.05

LOCATION: SWAN ROOM

IN SILICO ANALYSIS OF POTENTIAL VACCINE CANDIDATES FOR TRITRICHOMONAS FOETUS, THE CAUSATIVE AGENT OF BOVINE TRICHOMONIASIS

Eleanor Senior, University of Liverpool

Tritrichomonas foetus is an anaerobic flagellated protist and the causative agent of the venereal disease bovine trichomoniasis. This disease causes spontaneous abortions and, in some cases, infertility in cows and is responsible for decreased calving rates and milk production; infected animals are usually culled. Bovine trichomoniasis is therefore responsible for significant economic losses to farmers in several countries where the disease is endemic, including Australia, Brazil and the USA. Currently, there is no vaccine available that can prevent reinfection.

In order to identify potential vaccine candidates for this parasite, a reverse vaccinology approach was implemented. The Tritrichomonas foetus genome was sequenced on the PacBio platform(147Mb, N50=84,706), assembled using SMRTportal and then annotated using multiple automated processes including BRAKER, SNAP and BLAST2GO, integrated with transcriptomic data from both trophozoite and pseudocyst cell types, and improved through manual curation. Cell surface specific genes were identified using in silico prediction of signal peptides, transmembrane domains and GPI anchors.

In our T. foetus genome 84,706 genes have been identified, 1,607 of which contain a signal peptide and a transmembrane helix suggesting they are cell surface expressed and will be further examined as potential epitopes. Once a reduced set of genes has been produced, they will be recombinantly expressed and tested for their immunogenic potential.

We have produced the first fully-annotated T. foetus genome as the first step in a reverse vaccinology approach to this important livestock disease. Preliminary analysis of predicted cell surface proteins has resolved diverse transmembrane proteins as potential vaccine candidates.

NOVEL ANTIBIOTICS FROM ACTINOMYCETES TARGETING TEICHOIC ACID PG LIGASE

Shilpa Chatterjee, Centre for bacterial cell biology, Newcastle University

The emergence spread and rapid rise of antibiotic resistance is an alarming issue in the world today. Novel antibiotics are urgently needed to combat the rise of antibiotic resistant bacteria. The cell wall of the bacteria has been a very important source of targets for the development of antibiotics so far and many antibiotics in use today act on cell wall components. One overlooked component are wall teichoic acids which fulfil an important role in fundamental aspects of gram-positive physiology. In this study, genetically modified strains of the model system B.subtilis are used to examine their hypersensitivity to WTA inhibitors. A Prolific source of novel antibiotics are the filamentous soil bacteria Actinomycetes which produce the magnitude of clinically used antibiotics. In search of a novel WTA inhibitor, a library of over 2820 Actinomycetes extracts were screened in the previous project and seven hits were obtained. The validation and identification of these potential hits were at the core focus of this study. Media screening was conducted on both solid and liquid media to find compounds that are differentially active (wild type vs ETA inhibitor hypersensitive strains). Two Actinomycetes strains that produced well on both solid and liquid media were subsequently upscaled for cultivation in 15L

working volume bioreactors. Post purification of these two strains, a compound was identified via preliminary mass spectrophotometry analysis that shares the same accurate mass with two compounds in the literature, namely alisamycin and albofungin. Further work will focus on structure elucidation and target validation.

DRUGS ON THE BRAIN: AN IN VIVO MODEL FOR IMPROVING CRYPTOCOCCAL MENINGITIS THERAPEUTICS

Christopher Donaldson, Infection, Immunity & Cardiovascular Disease, Medical School, University of Sheffield

Cryptococcal meningitis (CM) is a devastating opportunistic infection primarily affecting the severely immunocompromised. This disease results in high mortality, particularly in resource-limited settings. The mainstay treatment of CM relies on two highly toxic fungicidal agents with relatively limited central nervous system (CNS) penetration, or, in resource-limited settings, only a fungistatic agent is used. The development of novel antifungals for CM must meet certain criteria – most critically, availability in resource-limited settings and high CNS penetration. We present two approaches to improving CM therapeutics; developing understanding of the pharmacokinetics of current treatments and mechanisms of CNS entry, and investigating currently available CNS-penetrating compounds in a repurposing study.

In vivo toxicity screens have been undertaken in zebrafish to establish safe doses of the current treatments. For this, we have determined known characteristics of the currently used antifungals and tested a range of concentrations in zebrafish. Flucytosine and fluconazole appeared to be safe, whereas amphotericin B induced severe toxicity at multiple different doses. We have generated two novel zebrafish models specifically for the investigation of CM, whereas previous models have focused on systemic infections. We are using these models to understand PK/PD related to CM, as they provide significant advantages over previous models. For example, we are currently using the fluorescent dye rhodamine 6G to investigate the effect of transporter and channel modulators on chemical transport into and out of the zebrafish larvae. Thus, we can show how our zebrafish CM model has significant potential in furthering our understanding of antifungal therapeutics.

HEROES AND VILLAINS: THE INTERACTIONS BETWEEN ANTIBIOTICS AND UROPATHOGENS TO MANAGE UTI

Aaron Ming Zhi Tan, Institute for Cell and Molecular Biosciences, Newcastle University

Urinary tract infections (UTI) are a growing public health problem, with high recurrence rates and increased antimicrobial resistance complicating clinical management. Uropathogenic *Escherichia coli* (UPEC) is associated with between 60-80% of UTI cases.

Here we describe the analysis of UPEC clinical isolates from the clinical trial AnTIC. AnTIC was an unblinded randomised controlled trial that compared antibiotic prophylaxis versus ad-hoc treatment to manage recurrent UTIs in clean intermittent self-catheterised patients. Analysis of the AnTIC microbiological records revealed that some prophylaxis antibiotic use selected against UPEC while other prophylaxis antibiotics and ad-hoc treatment favoured UPEC colonisation.

A key outcome of AnTIC was that rUTI frequency was lower among prophylactic patients. Our analysis argues that prophylaxis correlates with stable UPEC colonisation, when UPEC was isolated. This suggests a commensal-like role in preventing more pathogenic strains/species from colonising the urinary tract.

To investigate the evolution of antimicrobial resistance (AMR), we performed whole-genome sequencing on UPEC isolates from 20 ANTIC patients that acquired multi-drug resistant (MDR) UPEC during the trial. Phylogenetic analysis revealed that nine patients retained the same strain, arguing that in these cases MDR evolved rather than incidences of new MDR strain infections.

Our data provides a unique insight into the dynamics of microbial colonisation during

antibiotic use for a specific UTI at-risk group. Our analysis is therefore a foundation to develop alternative strategies to manage UTI risk in clean intermittent self-catheterised patients.