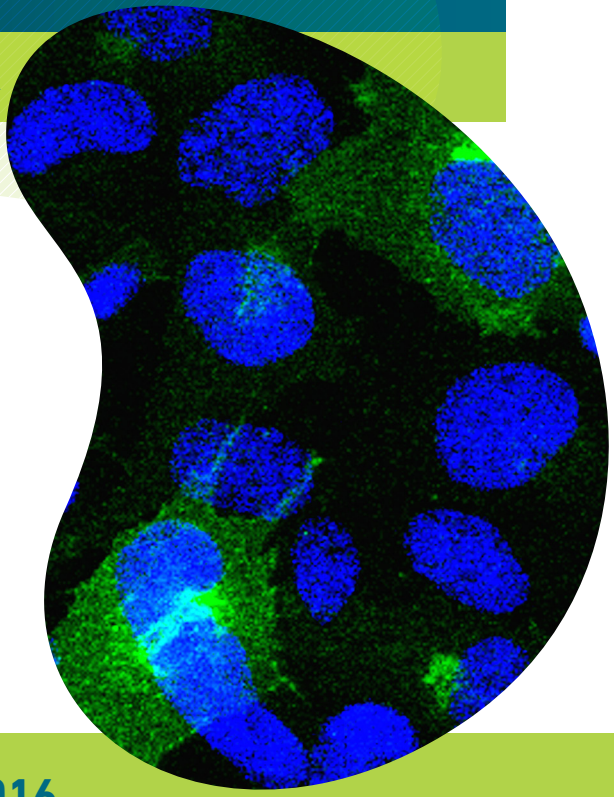
MICROBIOLOGY SOCIETY

Focused Meeting 2016 – Irish Division:

Host–Pathogen Interactions

Poster Abstract Book



30 JUNE–1 JULY 2016

Trinity College Dublin, Ireland

Focused Meeting 2016 – Irish Division: Host–Pathogen Interactions

Focused Meeting 2016 – Irish Division: Host–Pathogen Interactions

Poster Number: P01

Differential gene expression analysis of acute Ebola virus patients with fatal or non-fatal infections

Natasha Rickett[1], Sam Haldenby[2], Xuan Liu[2], Isabel Garcia Dorvial[1], Yongxiang Fang[2], Miles Carroll[3], Julian Hiscox[1]

[1]Infection Biology, University of Liverpool, Liverpool, UK, [2]Centre for Genomic Research, University of Liverpool, Liverpool, UK, [3]Public Health England, Porton Down, Wilts, UK

The recent outbreak of *Zaire ebolavirus* (EBOV) in West Africa was unprecedented in scale and caused by the most pathogenic species in the family *Filoviridae*. Here, RNA-sequencing was performed on 179 patient samples from the 2014-2015 outbreak in order to assess which genes, if any, are differentially expressed in fatal verses non-fatal EBOV infections. A number of genes were identified as fitting this criterion, including those associated with blood clotting, complement regulation and the acute phase response. These pathways, along with cell migration, were observed to be highly enriched in the samples of hospitalised individuals who eventually succumbed to infection compared to those who survived. Our RNA-seq data also allowed the employment of a machine learning technique in order to create a genetic profile for the average survivor. With the expression levels of ten genes we are able to predict patient outcome with an accuracy of 85%. The identification of these differentially expressed genes could illuminate some of the factors influencing patient outcome. This, along with the formation of a "survivor profile", could have clinical implications, both in terms of the development of novel therapeutics and the allocation of hospital resources.

Poster Number: P02

Development of a Fluorescent In-Situ Hybridisation (FISH) assay to detect vancomycin resistant Enterococcus faecalis in a novel biofilm model.

Michael Conwell, Patrick naughton, James Dooley

Ulster University, Coleraine, Northern Ireland, UK

Aim
To develop a FISH assay to detect the vanA gene of E. faecalis in a novel biofilm model.

Methods
Biofilm model - Vancomycin resistant E. faecalis (VRE) were grown in tryptone soy broth (TSB) to 2.5x109 CFU/ml. Cells were harvested, re-suspended and diluted 1:200 in PBS, TSB or TSB (vancomycin 10µg/ml). VRE were inoculated into 25µl gene frames, and biofilms were developed at 37oC for 24hrs.

FISH - Biofilms were washed with PBS and fixed in 97% ethanol for 5 mins. Fixed biofilms were incubated with fluorescein -labelled FISH probes specific to vanA for 2-24hrs at 50oC. A texas red probe targeted to E. faecalis 16s rRNA was used as a control.

Results
The gene frame method allowed for the reproducible assay of weak to moderate biofilm producing enterococci.

Control FISH probes to 16s rRNA gave a strong intracellular signal in cells grown both planktonically and in biofilm.

The vanA FISH probe demonstrated intercellular specificity, although in planktonic cells the staining pattern was diffuse and less intense than the 16s staining. In biofilm the vanA probe resulted in a significant increase in extracellular signal associated with bacterial extracellular polymeric substances. This was observed regardless of nutrient content used for biofilm development and it was not present in controls.

Conclusions
This study utilised gene frames® for the novel analysis of biofilm directly on microscope slides. FISH probes designed to specifically bind to the plasmid-associated vanA gene, demonstrate staining patterns not observed in planktonic cells.

Poster Number: P03

RNA-seq transcriptional profiling of PPD-b-stimulated peripheral blood from cattle infected with *Mycobacterium bovis*

Carolina C. Correia[1], Kirsten E. McLoughlin[1], Nicolas C. Nalpas[2], David A. Magee[1], John A. Browne[1], Kate E. Killick[1], H. Martin Vordermeier[3], Bernardo Villarreal-Ramos[3], Stephen V. Gordon[4], David E. MacHugh[1]

[1]Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Dublin, Ireland, [2]Proteome Center Tübingen, University of Tübingen, Tübingen, Germany, [3]Animal and Plant Health Agency, Weybridge, Addlestone, Surrey, UK, [4]UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland

Mycobacterium bovis infection, the cause of bovine tuberculosis (BTB), costs an estimated $3 billion to global agriculture annually. During the last decade, the maturation of high-throughput sequencing technologies coupled with well-annotated genome resources, has provided an unprecedented opportunity to gain a deeper understanding of host-pathogen interactions for many infectious diseases. Within this context, transcriptional profiling of the host immune response to *M. bovis* infection is a powerful approach for identifying host genes and cellular pathways important to disease pathology. For the present study, ten age-matched male Holstein-Friesian calves were infected endobronchially with *M. bovis* (~2,000 CFU). Peripheral blood samples were collected in duplicate at four time points (-1 wk pre-infection, +1 wk, +2 wk, and +10 wk post-infection) and used for a) an overnight stimulation with purified protein derivative of bovine tuberculin (PPD-b) at 37°C or b) a control overnight incubation at 37°C without PPD-b stimulation. After isolation of total RNA, poly(A)+ purified RNA was used to generate strand-specific RNA-seq libraries for high-throughput sequencing. Transcripts were quality checked, adapter and quality filtered, and then aligned to the *Bos taurus* reference genome UMD3.1.1. Following summarisation of gene counts, lowly expressed transcripts were removed prior to subsequent gene annotation and differential expression analyses. Results showed 929 differentially expressed (DE) genes at -1 wk pre-infection, 1,619 DE genes +1 wk post-infection, 1,170 DE genes at +2 wk, and 5,535 DE genes at +10 wk (compared to non-PPDb-stimulated group at each time point; FDR correction threshold ≤ 0.05).

Poster Number: P04

Diverse Responses of Campylobacter jejuni strains in Human and Chicken Intestinal Epithelial Cells.

Daniel John, Samuel Sheppard, Tom Wilkinson

Swansea University, Swansea, UK

Background: Campylobacter remains the major cause of gastroenteritis-associated food poisoning in the Western World causing a significant burden to health services. Campylobacter are particularly diverse, with a broad host range and significant genetic recombination within and between species. The response of chickens and humans to Campylobacter is dramatically different with the former able to tolerate particularly large doses (108 cfu) while the latter remains particularly sensitive (102 cfu).
Aim: To investigate this compelling difference, the inflammatory and cytotoxic responses of 131 Campylobacter strains from different sources were determined in chicken and human intestinal cells.

Method: Campylobacter isolates from multi-locus sequence typed collections were cultured on blood free selective medium and incubated at 42oC under a microaerophilic environment and their motility assessed. Standardised aliquots of Campylobacter strains were used to infect intestinal human HT-29 cells and chicken 8E11 cells prior to determination of inflammatory (qPCR) and cytotoxic (alamar blue) responses.
Results: Studies confirmed the flaA/B genes to have importance for Campylobacter motility. Human strains of Campylobacter induced higher inflammatory responses than strains isolated from chicken, wild bird or cattle as shown by significant increases in IL-8 and decreases IL-10 responses in HT-29 ($P \leq 0.01$) and 8E11 cells ($P \leq 0.05$). Analyses of cell viability after infection showed significant increases in cell toxicity by wild bird strains ($p \leq 0.01$). In addition cytotoxicity confirmed cdtA to have an important role in cytotoxicity ($p \leq 0.001$).

Conclusions: These results contribute to understanding the differential interactions of Campylobacter in humans and chickens.

Poster Number: P05

The nucleoprotein of Ebola-Makona interacts with HSP70 to promote protein stability

Isabel Garcia Dorival[1], Weining Wu[1], Stuart Armstrong[2], John N. Barr[3], Miles W. Carroll[4], Roger Hewson[4], Julian A. Hiscox[1]

[1]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [2]NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, UK, [3]School of Molecular and Cellular Biology, Faculty of Biological Science, University of Leeds, Leeds, UK, [4]Public Health England, Porton Down, Salisbury, UK

Ebola virus infection can result in severe disease and in some cases a lethal haemorrhagic fever. The infection is directed by seven viral encoded genes that encode nine viral proteins. By definition viruses are obligate intracellular parasites and require many aspects of host cell biology in order to replicate, assemble and subvert host cell anti-viral responses. Currently licenced antivirals are targeted against viral proteins to inhibit their function. However, experience with treating HIV and influenza virus demonstrates that resistant viruses can soon be selected. An emerging area in virology is to transiently target host cell proteins that play critical pro-viral roles in virus biology, especially for acute infections. This has the advantage that the protein being targeted is evolutionary removed from the genome of the virus. Proteomics can aid in discovery biology and identify cellular proteins that may be utilised by the virus to facilitate infection. This work focused on defining the interactome of the EBOV nucleoprotein and identified that cellular chaperones, including HSP70, associate with this protein to promote stability. Utilisation of a mini-genome replication system based on a recent Makona isolate demonstrated that disrupting the stability of NP had an adverse effect on viral RNA synthesis.

Poster Number: P06

Treatment and prevention of catheter related infections caused by *Staphylococcus aureus*

Siobhan Hogan[1], Eoghan O'Neill[0]

[1]Department of Clinical Microbiology, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 09, Ireland, [2]Department of Microbiology, Connolly Hospital,, Dublin 15, Ireland

Staphylococcal biofilms grown on implanted biomaterials, under in vivo conditions, are SaeRS-regulated and dependent on coagulase-catalysed conversion of fibrinogen into fibrin. This study has focused on the prevention of staphylococcal biofilm formation by blocking the coagulase mediated activation of pro-thrombin and the treatment of staphylococcal biofilm by digesting the fibrin matrix. Flow biofilm assays in biomimetic conditions were developed to assess the potential of a number of currently available anticoagulant therapeutics to prevent biofilm development. Both static and flow biofilm assays were employed to assess a number of fibrinolytic agents for the treatment of established *S. aureus* biofilm.

Three anticoagulants, Dabigatran (400nM), Argatroban (10µM) and Hirudin (25µg/ml), were found to be effective at preventing the development of *S. aureus* biofilm. A recombinant trypsin-like protease (TrypLE^{TM}) and fibrinolytic agents serrapeptidase, tranexamic acid, plasmin and nattokinase both proved effective in the disruption of established *S. aureus* biofilm. In conclusion anticoagulants targeting exosite one of prothrombin have been shown to be effective in the prevention of coagulase mediated biofilm and present a preventative option clinically for the prevention of *S. aureus* mediated biofilm infections. Fibrinolytic agents tested provide a potential treatment option for *S. aureus* mediated biofilm infections. Work is ongoing to assess the resistance profile and cytotoxic effects of these agents.

Poster Number: P07

Enhanced Natural Killer Cell Activity and Natural Resistance to Viral Infection amongst Irish Women Exposed to Hepatitis C Virus via Contaminated Anti-D Immunoglobulin

Mark Robinson[1], Ciara Keane[1], Margaret Needham[1], Clair Gardiner[1], Diarmaid Houlihan[2], Cliona O'Farrelly[1]

[1]School of Biochemistry & Immunology, Trinity College Dublin, Dublin, Ireland, [2]Liver Unit, St. Vincent's University Hospital, Dublin, Ireland

Natural resistance to viral infection could inform future therapeutic and vaccination strategies targeting hepatitis C virus (HCV). While adaptive immune responses to acute/chronic HCV infection are well studied, the mechanisms that allow some individuals to resist infection in the absence of adaptive immunity are unknown. This research is hampered by a lack of cohorts with defined pathogen exposure and documented evidence of infection. In Ireland, individuals exposed to HCV through contaminated anti-D immunoglobulin provide a unique opportunity to study mechanisms of resistance in a well-defined human cohort. This study profiled innate immune responses in a cohort of seronegative Irish women exposed to contaminated anti-D immunoglobulin (n = 16). Analysis of intracellular signalling following IFNα stimulation identified enhanced signalling exclusively in NK cells from the exposed seronegative group compared to matched unexposed controls (mean MFI fold change 2.81 vs 1.96, P-value = 0.003). At a functional level NK cells from exposed seronegative individuals had stronger IFNγ responses compared to matched unexposed controls (mean 50.9% vs 31.8%, P-value = 0.046). Our results describe a general enhancement of NK cell activity in exposed seronegative individuals who have resisted HCV infection and provides insights into the mechanisms of innate resistance to viral infection.

Poster Number: P08

Assessing the impact of treating commercial piglets with a 3rd generation cephalosporin injection on antimicrobial resistance of commensal faecal *E. coli*

Ana Vale[1], James Gibbons[1], Edgar Manzanilla[2], John Egan[3], Seamus Fanning[4], Laura Boyle[2], Finola Leonard[1]

[1]School of Veterinary Medicine University College Dublin, Dublin, Ireland, [2]Teagasc Pig Production Development Unit, Moorepark Research Centre, Fermoy, Ireland, [3]Central Veterinary Research Laboratory, Backweston Laboratory Campus, Celbridge, Ireland, [4]UCD Centre for Food Safety, School of Public Health, Physiotherapy and Population Science, Dublin, Ireland

Antimicrobial resistance (AMR) is a major human and animal health threat that is linked with antimicrobial use in both human and veterinary medicine. Third-generation cephalosporins (TGCs) are included in WHO and OIE lists of critically important antibiotics.

In Irish pig production TGCs are mainly used in piglets to treat infections such as meningitis. Faecal samples from 38 three-days old piglets (12 untreated piglets in control pens, 14 treated and 12 untreated piglets in treatment pens) from a commercial farm with no previous use of TGCs were investigated to evaluate the impact of a long-acting TGC injection (ceftiofur) on cefotaxime resistance in commensal *E. coli*. Faecal swabs were collected at 7 time-points over a 22 day period (D0 before ceftiofur, D1, D3, D7, D9, D15, D22). Additionally, faecal samples were collected from their dams (8 sows) at 6 time-points. Samples were screened for the presence of cefotaxime-resistant *E. coli* using TBX agar supplemented with cefotaxime according to EUCAST guidelines.

All *E. coli* isolates screened during the trial were cefotaxime-susceptible suggesting that single use of TGC on a naïve farm does not increase the number of cefotaxime-resistant *E. coli* detected.

Poster Number: P09

Investigating the role of surface proteins in *Staphylococcus aureus* skin infections.

Keenan Lacey, Joan Geoghegan, Rachel McLoughlin

School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland

Staphylococcus aureus is the most common cause of skin and soft tissue infections (SSTIs). Community acquired methicillin resistant *S. aureus* (CA-MRSA) infections are occurring with increasing frequency in healthy individuals and 90% of CA-MRSA infections present as SSTIs. The mechanisms of *S. aureus* virulence in these infections are incompletely understood.

To investigate the role of *S. aureus* cell wall-anchored (CWA) surface proteins during SSTIs, we generated sortase A (*srtA*), clumping factor A (*clfA*), clumping factor B (*clfB*), and protein A (*spa*) deficient mutants in the bioluminescent USA300 strain, LAC:*lux*. The strains were tested in a murine sub-cutaneous abscess model. Mice infected with LAC:*lux srtA, clfA* and *clfB* strains developed significantly smaller abscesses than those infected with the wild-type strain. *In vivo* bioluminescence was greatly reduced in mice infected with mutant strains compared to mice challenged with the wild-type strain and this correlated with lower bacterial burden in the abscess on day 3 and 6. Histological analysis revealed altered abscess architecture in LAC:*lux srtA* infected mice compared to wild-type infected mice.

These results indicate that CWA surface proteins of *S. aureus* are important for the establishment of skin infection and may contribute to the pathogenesis of *S. aureus* SSTIs.

Poster Number: P10

LPS and the NLRP3 inflammasome activator nigericin drive differential IL-1 and caspase expression in bovine endometrial cell populations

Paul Kelly[1], Kieran G. Meade[2], Cliona O'Farrelly[1]

[1]Comparative Immunology Research Group, School of Biochemistry & Immunology, Trinity Biomedical Sciences Institute, Trinity College, Dublin 2, Ireland, [2]Animal & Bioscience Research Department, Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Co. Meath, Ireland

Major changes in the uterine microbiome occur in all cows postpartum, resulting in inflammation associated with healthy involution. However, 30% of cows develop endometritis, a pathological uterine inflammation that compromises fertility. Here we investigate roles for endometrial epithelial and stromal cell populations in determining the switch from healthy to pathological inflammation and hypothesize that regulation of inflammasome activity is key.

Stimulation of uterine epithelial and stromal cells from 6 animals with nigericin in combination with LPS resulted in IL-1α and IL-1β expression in a time dependent manner, with mRNA levels peaking after 6 hours. Levels of IL-1β mRNA were significantly higher in stromal cells (mean fold change=86.9, p=0.0062) than in epithelial cells (mean fold change=1.7) at 6 hours. This was verified at a protein level using an ELISA. However, expression of caspase 8 and 13 mRNA was higher in epithelial cells (caspase-8 mean fold change=11.8, caspase-13 mean fold change=7.2; n=3) than in stromal cells (caspase-8 mean fold change=2.5, caspase-13 mean fold change=1.5; n=5) at 6 hours.

The data suggests that bovine endometrial cell populations play divergent roles in uterine inflammation through differential regulation of IL-1β production. Identifying the molecular mechanisms responsible for differential inflammasome activation will define its role in uterine inflammation, ultimately leading to novel therapeutic interventions for bovine endometritis.

Poster Number: P11

Identification of endemic avian viruses causing poultry production problems via next-generation sequencing.

Ryan Devaney[1], S.Louise Cosby[2], Caroline Meharg[1], Victoria Smyth[2]

[1]Queen's University Belfast, Belfast, UK, [2]Agri-Food and Biosciences Institute, Belfast, UK

Runting-stunting syndrome (RSS) is a production disease affecting broiler chickens and is considered to be multi-factorial. Factors influencing the disease include bacterial infection, age, feed quality, immune status, and viral infection arising from a community of viruses (virome). Previous investigations into the enteric viruses associated with poultry have been limited to culture-dependant methods and assays targeting previously known viruses. This project has established a virological profile associated with RSS using next-generation sequencing (NGS) which has been used to compare data between affected flocks and unaffected flocks. Preliminary sequencing results identified the presence of 20 RNA and DNA virus families comprised of 31 distinct viral genera and 7 unclassified categories associated with affected and unaffected samples collected from multiple UK farms. These include chicken astrovirus (CAstV), avian nephritis (ANV) 1 & 2, chicken megrivirus, chicken parvovirus, and sicinivirus 1; a novel picornavirus. Additionally, evidence has been found indicating the presence of novel organisms; subject to further analysis. A longitudinal sequencing study was completed identifying a range of viruses associated with good and poor performance farms over a 17 day growth period. These include members of the viral families *Astroviridae, Caliciviridae, Retroviridae, Picornaviridae,* and *Parvoviridae*. Further quantitative studies have been undertaken and are required to understand the temporal expressions of these viruses and their overall role in the disease. High-throughput next generation sequencing in this study presents a viable technique for the discovery and characterisation of the complex viral communities present in the gastrointestinal tract of disease-affected and unaffected broiler chickens.

Poster Number: P12

Presence of Quorum Sensing Deficient *Pseudomonas aeruginosa* Isolates in Patients with Cystic Fibrosis

Syed Ahmed, Michelle Rudden, Karl Smith, James Dooley, Ibrahim Banat

Ulster University, Coleraine, UK

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen associated with Cystic Fibrosis (CF) lung disease. During chronic lung colonization *P. aeruginosa* undergoes a characteristic evolutionary adaptation that enables the bacterium persist in the dynamic lung environment for long periods of time, often resulting in quorum sensing (QS) mutants in the regulatory genes like *lasR* and *rhlR.*

Results: We screened clinical isolates from CF patients for characteristic QS traits. We isolated several strains deficient in these QS traits and analysed the gene expression profile of a selected isolate (PA80) for regulatory QS associated genes and virulence associated genes. Using a previously validated RT-qPCR assay for *P. aeruginosa* gene expression profiling, we have shown that clinical isolate PA80 is deficient in gene expression of the QS regulatory gene *lasI,* responsible for the synthesis of the signal molecule 3-oxo-C12-HSL. This resulted in >30-fold downregulation of the QS controlled virulence genes *lasAB* and *rhlABC*, which produce virulence factors elastase and rhamnolipids respectively. Transcriptional analysis also revealed that *rpoS,* a sigma virulence factor, was significantly downregulated in PA80 relative to wild-type levels in PAO1. The transcriptional profiles show the phenotypic diversity of *P. aeruginosa* populations in the CF lung environment and its adaptive ability to reduce acute virulence during chronic infection as an essential survival mechanism.

Conclusion: Understanding the evolution of *P. aeruginosa* during chronic prolonged infection will be essential for developing novel therapeutic strategies such as targeting long term adaptive mechanisms or QS coordination of the whole population compared to conventional antibiotic therapy which does not work.

Poster Number: P13

Defective production of cell wall anchored proteins and secreted virulence factors in teichoic acid deficient mutants of Staphylococcus aureus

Joana Carvalho, Joan Geoghegan, Timothy Foster

Trinity College, Dublin, Ireland

Wall teichoic acid (WTA) is an anionic polyol phosphate polymer that is covalently attached to peptidoglycan. WTA plays an important role in cell growth, division, morphology, virulence and resistance to β-lactam antibiotics in Staphylococcus aureus. The sortase A enzyme mediates covalent attachment of cell wall-anchored (CWA) proteins to the pentaglycine cross-bridge of peptidoglycan so that the proteins are displayed on the surface of the bacterium.
Here, we investigate the CWA proteins such as protein A, clumping factor A and B are present on the surface of WTA-deficient mutants of S. aureus at the same level as on wild type bacteria.

The enzyme TarO initiates the assembly of WTA. Here, isogenic tarO-deficient mutants of methicillin sensitive and methicillin resistant strains of S. aureus were generated. Lower levels of the CWA proteins were detected in WTA-deficient tarO mutants compared to the corresponding parental strains. As a consequence, WTA-deficient mutants of S. aureus adhered poorly to ligands such as loricrin, fibrinogen and immunoglobulin G. The wild-type phenotype was restored by complementation with a plasmid expressing tarO. Reduced transcription of protein A and clumping factor B was detected and no changes were detected in clumping factor A, alpha toxin and membrane proteins transcription.

Unveiling the role of WTA in the display of CWA proteins in S. aureus will lead us to understand the role of these key players in colonisation/pathogenesis leading to a better understanding of possible drug targets to combat S. aureus infections.

Poster Number: P14

IL-29 has potential as a novel prophylactic drug against respiratory syncytial virus.

Lindsay Broadbent, Remi Villenave, Lyndsey Ferguson, Michael Shields, Ultan Power

Queen's University, Belfast, UK

Respiratory syncytial virus (RSV) is the principal cause of severe lung disease in infants. Innate immune responses to RSV infection are poorly understood. We exploited our well-differentiated primary paediatric bronchial epithelial cell model (WD-PBECs) to study IL-29 (IFNλ1) responses to RSV infection. We demonstrated that RSV infection of WD-PBECs induced an antiviral response, mediated in part by secreted factors, capable of reducing Sendai virus growth kinetics. We now show that conditioned basolateral medium from RSV-infected WD-PBECs (CMRSV) also reduces RSV growth. Using an IL-29 neutralising antibody we demonstrated that IL-29 was implicated in the antiviral activity associated with CMRSV. Therefore, we assessed the potential of IL-29 as a prophylactic or therapeutic drug against RSV. Apical pre-treatment of WD-PBECs with IL-29 significantly attenuated RSV growth kinetics but basal pre-treatment did not. Neither apical nor basal treatment of WD-PBECs with IL-29 following RSV infection altered virus growth kinetics. Interestingly, IL-28RA, a component of the IL-29 receptor, was located exclusively on the apical surface of WD-PBECs. Our data suggest that IL-29 has potential as a novel aerosolised prophylactic drug to help combat RSV spread.

Poster Number: P15

Identification and Phylogentic analysis of Novel Porcine Parvoviruses, Cyclovirus and Stool-Associated Virus in Mesenteric Lymph Node and Faeces Samples from Pigs in Northern Ireland

Naomi Magowen[1], Natalie Brush[1], Paula Lagan[3], PJ Collins[3], Helen O'Shea[2], S. Louise Cosby[3], Mark Mooney[1], John McKillen[3]

[1]Queen's University Belfast, Belfast, UK, [2]Cork Institute of Technology, Cork, Ireland, [3]Agri-Food and Biosciences Intitute, Belfast, UK

The control of viral diseases is an ongoing battle for pig farmers to prevent the economic losses associated with mortality and morbidity. Horizon scanning and monitoring of emerging threats is key to this control. Porcine parvovirus (PPV)1 is associated with reproductive failure in sows but in recent years a wide variety of parvoviruses have been identified, of unknown aetiology. Cyclovirus (CyCV) and porcine stool-associated circular viruses (PoSCV) are both circular single stranded DNA viruses, again of unknown disease association.121 porcine mesenteric lymph nodes (MLN) from veterinary submissions and 135 faeces samples from healthy slaughter age pigs were collected in Northern Ireland. PCR positive samples were confirmed by melting curve analysis and gel electrophoresis. MLN samples; 10/121 (8.3%) were +ve for PPV5, 1/121 (0.8%) was +ve for PPV6 and 0/121 (0%) were +ve for CyCV and PoSCV. Faeces samples; 23/135 (17%) were +ve PoSCV. Phylogenetic analysis was carried out on 5 PPV and 1 PPV6 capsid sequences. Resultant BLAST search and phylogenetic trees confirmed the sequences as PPV5 and PPV6 and PoSCV to belonging to group 3. Therefore, small DNA viruses were found to be ubiquitous in pig herds. PPV5, PPV6 and PoSCV were detected in this study while CyCV was not. The significance of these novel viruses remains unknown in terms of their potential to cause disease. They may be non-pathogenic, could function as co-factors in polymicrobial diseases or cause subclinical effects resulting in reduced performance. All have potential to mutate into more virulent forms and require ongoing monitoring.

Poster Number: P16

Phylogenetic Analysis of Porcine Circovirus type 2 (PCV2) Strains in Northern Ireland from Current and Archival Samples

Natalia Zaykalova[1], Paula Lagan[2], Michael McMenamy[2], S. Louise Cosby[2], Mark Mooney[1], Gordon Allan[1], John McKillen[2]

[1]Queen's University Belfast, Belfast, UK, [2]Agri-Food and Biosciences Institute, Belfast, UK

Porcine circovirus type 2 (PCV2) is the causative agent of the post weaning multisystemic wasting syndrome (PMWS). PMWS is a damaging disease that affected the pig industry worldwide. PCV2 can be divided into four genotypes known as PCV2a, b, c and d. It has been reported in several countries that a shift of prevalence from PCV2b to PCV2d is ongoing. Some reports of PCV2d associated wasting disease in vaccinated animal have been published but any role of PCV2d in vaccine failure needs further research. The objectives of this study were to carry out a phylogenetic evaluation of the currently circulating strains of PCV2 in Northern Ireland. These were also compared to archival sequences from the Island of Ireland in order to estimate shifts in genotype. Twenty two PCV2 positive mesenteric lymph node (MLN) samples were collected between 2011 and 2015. The open reading frame 2 (ORF2) gene, coding for the viral capsid, was amplified by PCR, sequenced and phylogenetic trees produced. An additional 28 archival sequences dating from 1997 to 2006 collected prior to the introduction of PCV2 vaccination were also analysed. The analysis showed a shift away from PCV2a to PCV2b. These findings are consistent with a previous study which confirmed that genotype shift to PCV2b in NI occurred around 2003. There is no evidence of genotypes PCV2c or d in NI pigs. However, close monitoring of the evolution of this virus is important, in relation to vaccine-induced selective pressures which could give rise to infection in vaccinated animal.

Poster Number: P17

Respiratory syncytial virus infection and the role of an innate anti-viral protein

Helen Groves[1], Lindsay Broadbent[1], Kyra Kerkhofs[1], Hong Guo-Parke[1], Martin Moore[2], Michael Shields[1], Ultan Power[1]

[1]Queens University, Belfast, UK, [2]Emory University, Atlanta, USA

Respiratory syncytial virus (RSV) causes severe disease in 1-3% of infants annually. Reasons for the broad range of symptom severity in infants remain unclear. A microarray study was undertaken comparing transcriptome responses in well-differentiated primary nasal epithelial cell cultures (WD-PNECs) derived from cohorts of infants with histories of mild or severe RSV disease. Gene x was downregulated at baseline in children with a history of severe versus mild disease and was unaffected by RSV infection. Recombinant protein X reduced RSV infection in well-differentiated primary paediatric bronchial epithelial cells (WD-PBECs) following pre-treatment at 4oC. Pre-treatment of BEAS-2B cells and WD-PBECs with a neutralising antibody targeting X resulted in increased RSV infection. To determine whether protein X affected RSV entry, BEAS-2B cells were incubated on ice and infected with RSV followed by treatment with protein X. Following shifting to 37oC for 10 min, the cells were washed with citrate buffer (pH 3.0). RSV infection was reduced in protein X-treated cultures versus controls, indicating it attenuates RSV entry. Our data suggest protein X acts as an innate antiviral molecule, interfering with binding and/or entry of RSV to cells. The relative endogenous quantity in infant airways may contribute to susceptibility to severe RSV disease.

Poster Number: P18

Well-differentiated primary nasal epithelial cell (WD-PNEC) cultures derived from newborns: an exciting opportunity to study airway innate immune responses in high risk groups

Helen Groves, Patricia Coulter, Hong Guo-Parke, Lindsay Broadbent, Michael Shields, Ultan Power

Queens University, Belfast, UK

Respiratory syncytial virus (RSV) is the commonest cause of severe lower respiratory tract infection in infants under two-years worldwide. Young and premature infants are at greater risk of severe RSV-related disease. Little is known about the airway epithelial responses to pathogens in these groups. We aimed to establish and characterise WD-PNEC cultures derived from term and preterm infants within hours/days of birth in terms of morphology and responses to RSV infection.
Interdental brushes were used to obtain nasal epithelial cells from term and preterm infants within 48 h of birth. Infants with cystic fibrosis were recruited at the time of diagnosis on newborn screening (6-8 weeks old). Morphologically and physiologically authentic WD-PNECs were successfully generated from the cells, as characterised using light microscopy and immunocytochemistry. Newborn WD-PNEC cultures had extensive cilia coverage and mucous production. Robust RSV growth kinetics were evident following infection of newborn derived WD-PNECs with higher RSV titres seen in WD-PNECs derived from term versus preterm neonates. These newborn WD-PNECs represent a unique opportunity to study differential airway epithelium innate immune responses to RSV in very early in life.

Poster Number: P19

Relative cytopathology and transcriptional profile in well-differentiated paediatric primary nasal epithelial cell cultures (WD-PNECs) derived from cohorts of infants with histories of mild or severe RSV disease

Hong Guo-Parke[1], Olivier Touzelet[1], Isobel Douglas[2], Remi Villenave[1], Liam Heaney[1], Peter Coyle[3], Michael Shields[1], Ultan Power[1]

[1]Queens University Belfast, Belfast, UK, [2]The Royal Belfast Hospital for Sick Children, Northern Ireland, UK, [3]3The Regional Virus Laboratory, Belfast Trust, Northern Ireland, UK

One to 3% of infant birth cohorts are hospitalised annually following RSV infection, while most infants have only mild symptoms. To address airway epithelial cells/RSV interaction in disease severity, we exploited RSV-infected well-differentiated paediatric primary nasal epithelial cell cultures (WD-PNECs) derived from cohorts of infants with histories of mild or severe RSV disease. A microarray study was also undertaken to compare transcriptome profiles in these cultures. The cohorts were indistinguishable in terms of ciliated and goblet cell content and tight junction integrity. RSV infection was restricted to apical ciliated cells with no noticeable damage to cultures from either cohort. Virus growth kinetics and syncytia formation were also similar in both. Interestingly, apical cell sloughing and apoptosis was slightly increased in mild, but not severe WD-PNEC cultures, relative to uninfected controls. Pro-inflammatory responses, such as RANTES, IP-10, TRAIL and MMP were similar following infection, although IL-29 secretions were diminished in the severe cohort. Our data suggest that there are few substantial differences of RSV host responses that might explain the differential disease severity between cohorts. However, differential transcriptome profiles and RT-qPCR revealed genes with diminished expression in severe compared to mild WD-PNECs suggesting possible biomarkers of susceptibility to severe RSV disease.

Poster Number: P20

The development of respiratory epithelium innate immune responses to Respiratory Syncytial Virus in infants with Cystic Fibrosis

Patricia Coulter[1], Helen Groves[1], Lindsay Broadbent[1], Michael D. Shields[1], Ultan F. Power[1]

[1]Queens University, Belfast, UK, [2]Royal Belfast Hospital for Sick Children, Belfast, UK

RSV infection is the major cause of severe lower respiratory tract disease in young infants. Evidence suggests that cystic fibrosis (CF) is associated with severe lung disease following RSV infection. We and others previously demonstrated that airway epithelium is the primary target of RSV infection. However, little is known about the impact of RSV infection on CF airway epithelium. To address this, we aimed to establish well-differentiated primary nasal epithelial cell cultures (WD-PNECs) from recently diagnosed CF infants to study RSV cytopathogenesis in CF airway epithelium. CF WD-PNECs were successfully generated from 2 infants (8 and 9 weeks old) and characterised by light and fluorescent microscopy. These CF WD-PNECs had goblet cells that secreted thick dry apical mucous, consistent with *in vivo* observations. Good cilia coverage was also evident, although cilia beat frequencies appeared lower than those evident in WD-PNECs from healthy neonates. Preliminary data demonstrated RSV growth kinetics that were similar to those evident in WD-PNECs from healthy neonates, with peak virus titres evident at 72-96 hpi. Our preliminary data suggest that this model provides an exciting opportunity to elucidate the cytopathogenic, inflammatory and molecular consequences of RSV infection of airway epithelium derived from very young CF infants.

Poster Number: P21

A novel antiviral compound inhibits a range of enveloped viruses

Nicola F. Fletcher[1,2], Michael Folan[1], Claire Shannon-Lowe[2] and Alan W Baird[1]

[1]*Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland*
[2]*Cancer Sciences Centre, University of Birmingham, Birmingham B15 2TT, UK*

We evaluated a novel compound based on an emulsion of free fatty acids (ML:8) for its ability to inhibit a range of viral infections in vitro. Using physiologically relevant skin, tonsil and lung airway models, ML:8 treatment completely inhibited Epstein-Barr virus, measles virus and herpes simplex virus (HSV-1), together with Ebola, Lassa and vesicular stomatitis virus pseudoparticles, in a concentration- and time-dependent manner. Inhibition of viral infection by ML:8 occurred without detectable cellular toxicity and there was no evidence of development of viral resistance over time when cells were passaged in the presence of ML:8. ML:8 treatment had no effect on non-enveloped norovirus replication in macrophages or neuronal cells, indicating that its mechanism of action may be via disruption of the viral envelope. We have identified a novel antiviral compound based on an emulsion of free fatty acids that may have applications for the prevention and treatment of a diverse range of enveloped viruses.

Poster Number: P22

The morphology of influenza A virus particles does not affect the capacity of viral particles to travel through the air.

Kieran Dee[1], Ruth A Elderfield[2], Wendy S Barclay[2], Kim L Roberts[1]

[1]*Department of Microbiology, Moyne Institute of Preventive Medicine, School of Genetics and Microbiology, Trinity College Dublin, Dublin 2, Ireland*
[2]*Imperial College London, Faculty of Medicine, Division of Infectious Disease, Norfolk Place, London W2 1PG, UK*

Influenza A virus particles found in clinical isolates are pleomorphic. This phenotype is lost when the isolates undergo passage in either embryonated chicken eggs or MDCK cells, where the vast majority of virions become uniformly spherical. This suggests that pleomorphic particles confer an advantage *in vivo*, while a spherical phenotype is more advantageous *in vitro*. By introducing a single nucleotide substitution to the M gene of H3N2 A/Victoria/3/1975 we have changed the shape of the virions from a pleomorphic to a spherical phenotype. The mutant virus with spherical-only virions had a replicative advantage in MDCK cells and reduced neuraminidase activity. We used a novel *in vitro* transmission system to assess the relative capacity for pleomorphic or spherical populations of virus particles to travel through the air. Our results showed that there was no statistical difference between the two variant virus populations in their ability to travel through the air in a closed system.

Poster Number: P23

Plasminogen capture by the cell wall-anchored protein FnBPB of *Staphylococcus aureus*

Giampiero Pietrocola[1], Giulia Nobile[1], Valentina Gianotti[1], Marta Zapotoczna[2], Timothy J. Foster[2], Joan A. Geoghegan[2] and Pietro Speziale[1]

[1]Department of Molecular Medicine, Unit of Biochemistry, Pavia, Italy, [2]Department of Microbiology, Moyne Institute of Preventive Medicine, School of Microbiology and Genetics, Trinity College Dublin, Ireland.

The surface of the Gram-positive pathogenic bacterium *Staphylococcus aureus* is decorated with cell wall-anchored (CWA) proteins. Like several other invasive pathogens *S. aureus* can capture plasminogen (PLG) from human plasma where it can be converted to plasmin by host plasminogen activators or by endogenously expressed staphylokinase. A mutant defective in the sortase enzyme responsible for anchoring CWA proteins to the *S. aureus* cell surface captured 10-fold less PLG compared to the wild type indicating that one or more CWA proteins is involved. Analysis of PLG binding by purified recombinant CWA proteins indicated that FnBPA and FnBPB could recognize the host protein. Plasminogen captured on the surface of *S. aureus* or *Lactococcus lactis* expressing FnBPB could be activated to the potent serine protease plasmin by staphylokinase and tissue plasminogen activator and degraded host proteins such as fibrinogen. The binding domain on PLG was localized to kringle 4 while on FnBPB the PLG binding site involved two lysine rich regions within subdomain N3. Coating the bacterial cell surface with a host protease could aid pathogenesis of invasive *S. aureus* infection by destroying tissue and degrading blood clots.

Charles Darwin House, 12 Roger Street, London, WC1N 2JU, UK
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