Title: Virological and drug resistance outcome of switching HIV-1 positive patients on suppressive second-line therapy to ritonavir-boosted darunavir monotherapy in Cameroon.

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Abstract

Objective: To analyse the virological and drug resistance outcomes of switching HIV-1 positive patients established on suppressive second-line antiretroviral therapy (ART) to maintenance monotherapy of ritonavir-boosted darunavir (DRV/r) in Yaoundé, Cameroon.

Methods: A total of 120 patients were randomised 2:1 to switch to DRV/r monotherapy (800/100mg OD) for 48 weeks [n=81] or continue standard triple ART with 2 NRTIs+LPV/r [n=39] for 24 weeks. Patients attended study-visits at weeks 4, 12, 24, 36 and 48. Adherence was measured using pill-count and visual analogue scale (VAS) during visits. Virological failure was defined as plasma viral load >400 copies/ml on 2 measurements or last available measurement and treatment failure was defined as early discontinuation of DRV/r monotherapy. Drug resistance was evaluated using Sanger and NGS in blood cells at study entry and plasma samples at time of viraemia. This analysis evaluated outcomes in the DRV/r arm.

Results: Among 81 patients (60% females, median age: 42.8 years, median CD4 count: 425 cells/mm3) 22/81 had treatment failure of which 16 had virological failure. The median viral load at time of failure was log10 3.5 copies/ml. A further 12 patients did not meet the definition of virological failure but experience 1(n=9) or 2(n=3) episode of viraemia between 77 and 1220 copies/ml. In the multivariate analysis, virological failure was independently predicted by adherence measured by VAS. Genotyping did not show presence of resistance to DRV at study entry and time of failure.

Conclusion: In this study, the efficacy of switching HIV-1 patients to maintenance DRV/r in Cameroon was to similar studies in western cohorts with was no emergence of resistance to protease inhibitor.
Title: Aetiology of Central Nervous System Infections in Adults in Yogyakarta, Indonesia: A Three-Year Prospective Hospital-Based Study

Authors: Bardatin Lutfi Aifa, Janet Flatley, Tessa Prince, Sekar Satiti, Tom Solomon, Michael J Griffiths

Abstract

Background: Central nervous system (CNS) infections, such as meningitis and encephalitis, remain life-threatening, especially in developing countries. Pathogen diagnosis is essential to guide appropriate treatment. However, the aetiology of CNS infection, in much of Indonesia, including Yogyakarta, is incompletely understood.

Methods: A prospective hospital-based study was conducted in adults at Dr Sardjito Hospital, a large tertiary referral hospital for Yogyakarta, between February 2015 and January 2018. In addition to routine microbiology culture, we employed systematic testing of cerebrospinal fluid (CSF), including pathogen-specific PCR and antibody tests to identify pathogens.

Results: We recruited 196 suspected CNS infection patients (CSF obtained; n=141). Pathogens were detected in 39 cases; three by routine culture and 46 by systematic testing (two detected by both, 8 cases had 2 pathogen). Pathogens detected by systematic testing included: Mycobacterium tuberculosis (n=23), Orientia tsutsugamushi (n=13), Cryptococcus neoformans (n=3), Streptococcus pneumoniae (n=3), Herpes simplex virus type-2 (n=2), Varicella zoster virus (n=1), Dengue virus (n=1).

Conclusion: Introduction of systematic pathogen-specific PCR and antibody testing of CSF at the local laboratory increased pathogen detection by 26% (from 2% to 28%) in a large regional Indonesian hospital. For the first time, scrub typhus has been linked to CNS infection in Indonesia. This knowledge offers to inform clinical management of CNS infections. For example, through dissemination of results, clinicians will have a higher index of suspicion for treating mycobacteria and scrub typhus. Longer-term, uptake of systematic CSF testing will facilitate more targeted patient treatment and improve patient care.
Title: Immune kinetics in the turbinate and trachea of vaccinated and unvaccinated broiler chicks following IBV M41 challenge

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Abstract

Infectious bronchitis virus (IBV) is a highly contagious pathogen that causes respiratory, renal and reproductive diseases in chickens. Upper respiratory tissues are important, as it is typically the first site of infection and replication. This study aimed to examine the viral load and innate immune responses in turbinate and trachea tissue from vaccinated and non-vaccinated broiler chickens experimentally challenged with virulent M41. Day-old broiler chicks were vaccinated via the oculonasal route, and at 21 days post vaccination chicks were challenged with either virulent IBV M41 or sterile water. The IBV viral load and host gene expression were examined by real-time PCR and immunohistochemistry. At five days post-challenge (dpc), the viral RNA load in the turbinate was significantly higher than tracheal tissues in the control challenged group. Results showed significant high levels of mRNA gene expression of toll-like receptor 3 (TLR3) and melanoma differentiation-associated protein 5 (MDA5) in turbinate and trachea tissue of challenged groups compared to the control group. Chicken interferon beta (IFN-β) mRNA expression was significantly up-regulated in the tracheal tissues of vaccinated-control and vaccinated-challenge compared to the control chickens. Also, there was an upregulation of pro-inflammatory cytokine (Interleukin-6, IL-6) in the tracheal tissue. Our findings revealed that host gene signatures and cytokine responses could play a critical role in resisting IBV, especially for up-regulation of TLR3, MDA5, IFN-β and IL-6 mRNA expression.
Title: The affect of Human Papilloma Virus (HPV) 16 and 18 Long Protein Virus Antigens and HPV 16-E6 Tumor Peptide Antigen on Human Nasopharynx Associated Lymphoid Tissue (NALT) in Children

Authors: Alrusayyis, Fadiyah, Zhang, Qibo

1. Institution of Infection and Global Health

Abstract

Background: Human Papilloma Virus (HPV) is double strand DNA virus small in size and cause wide range of diseases from warts to cervical cancer. Oropharynx squamous cell carcinoma (OPSCC) is a part of head and neck squamous cell carcinoma (HNSCC) the sixth most cancer worldwide. Studies on HNSCC indicate that persistent infection with Human Papilloma virus type 16 is a dominant risk factor of development OPSCC. In the last three decades the prevalence of OPSCC associated with HPV showed significantly increased from 40.5% before 2000, and 72.2% between 2005 and 2009 in developed countries.

Aim: This study is aimed to assess the effect of HPV type 16 and 18 vaccine antigens and HPV16 E6 tumor peptide antigen in human nasopharynx associated lymphoid tissue.

Method: Children tonsils tissues were collected under tonsillectomies. The tonsil tissue were processed to isolate the mononuclear cells (MNC) and stimulate by HPV16, HPV 18 and HPV16 E6 antigens to evaluate the proliferation of effector T cells (CD4+ and CD8+) by using proliferation assay and stain the cells against CD4 and CD8 cell’s markers.

Result: In vitro, specific CD4+ T cells were induced following the stimulation of MNC by HPV 16 vaccine antigen in 2 of 3 children samples. However, no significant increase in the proliferation of CD4 and CD8 was observed to both HPV -18 and HPV16 E6

Conclusion: Our findings showed that, HPV 16 long protein vaccine antigen induces HPV 16 specific CD4+ T cells in vitro and immunogenic more than other antigens used in this study. It is possible that some of these young children more likely to expose to HPV 16 virus. Moreover, the HPV 16 virus antigen is expansion the frequency and activity of HPV 16 specific CD4+ cells.
Title: Interleukin-8 is a major factor in inducing Neutrophil Extracellular Traps (NETs) in sepsis

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Abstract

Background: Neutrophils provide the first line of defence against bacterial infection. Neutrophil extracellular traps (NETs) formation is a novel mechanism by which neutrophils immobilise and kill pathogens and are increase in critical illnesses such as sepsis. Recent studies highlight the harmful side effects of NETs by providing a scaffold for thrombosis, exposing cytotoxic histones and occluding the microcirculation, which ultimately leads to multiple organ failure (MOF). However, the mechanism leading to increased NETs formation in critical illness remains poorly understood.

Methods: A prospective cohort of 341 consecutive adult ICU patients were recruited. NETs were measures in the admission blood samples and correlated with daily clinical measurements and 27 circulating cytokines. Cecal ligation puncture (CLP) sepsis mice model complemented clinical observations.

Results: NETs are significantly increased in patients with sepsis compared to other critical illnesses. NETs formation was significantly associated with daily Sequential Organ Failure Assessment (SOFA) scores and high levels of NETs independently predicted 28-day mortality. Interleukin (IL)-8 levels were associated with NETs formation in critically ill patients. Specifically inhibiting IL-8 signalling using Reparixin inhibited NETs formation in patient samples. Therapeutic blocking IL-8 signalling in a septic mouse model protects against lung injury, attenuates NETs surrogate markers in lung tissues and improved survival rates.

Conclusion: We identify IL-8 as a major driving factor of NETosis in critical ill patients. Therapeutically, targeting IL-8 reduced NETs-induced organ injury and increases survival rates in mice with sepsis. This could provide a novel therapy in improving clinical outcome in critically ill patients.
Title: Faecal shedding of Rotarix® vaccine virus and mucosal immunity to rotavirus in a cohort of vaccines in the UK

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Abstract

Sequential faecal samples from twelve infants vaccinated with Rotarix® were collected throughout their vaccination period. Vaccine RNA shedding and total and rotavirus-specific copro-IgA were quantified in 17-45 samples/infant.

Viral RNA was extracted from faecal suspensions, reverse-transcribed and quantified using a vaccine-specific NSP2 qPCR (limit of detection, 10³ copies/g). Total copro-IgA was measured using a commercial ELISA kit and rotavirus-specific copro-IgA using an in-house ELISA.

Maximum shedding of 10⁹ copies/g was observed, with peaks at days 2 to 15 post dose 1. Lower amounts were detected after dose 2 and none after a year of vaccination. Rotavirus-specific copro-IgA was detected in 4/11 infants at pre-vaccination*, 7/12 infants after first dose, 5/12 infants after second dose and 3/8 infants after a year**, ranging from 10⁰-3000 µg/g.

While Rotarix® is reported to contain 10⁶ CCID₅₀/mL, we quantified stocks as 2-3 log₁₀ higher, suggesting significant amounts of non-infectious virus. Viral loads in stool fluctuated with time of shedding and were within the range of wild-type infections (10²-10¹⁰ copies/g), suggesting active replication in all infants, particularly at later time points; early shedding is likely the inoculum. High pre-vaccination specific copro-IgA levels in three infants are likely to originate from maternal antibody*** or wild type infection. Specific copro-IgA levels after vaccinations were high at time points of viral load control in most infants and vice versa.

* pre-vaccination sample not available for one infant

** after-a-year sample not available for four infants

*** breastfeeding information not available yet
Title: A genomics study of multidrug-resistant Pseudomonas aeruginosa strains isolated in a hospital in Thailand leads to discovery of a novel group of megaplasmids carrying a high diversity of antibiotic resistance genes

Authors: A Cazares¹, MP Moore¹, M Grimes¹, I Kukavica-Ibrulj², P Pongchaikul³, P Santanirand³, RC Levesque³, J Fothergill², C Winstanley¹

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Abstract

The spread of antibiotic resistant bacteria represents a major health threat worldwide. Among the pathogens recently recognized by the WHO as priority to fight antibiotic resistance, Pseudomonas aeruginosa occupies one of the highest positions in the critical tier. Extensive research has linked P. aeruginosa resistance to a variety of chromosomal genetic factors, yet, given the notable genomic diversity of this bacteria, novel resistance elements are expected to be discovered. Here we conducted a genomics study of a set of multidrug-resistant P. aeruginosa isolates from Thailand to unravel the genetic determinants contributing to their resistance.

A panel of P. aeruginosa strains isolated in a hospital in Bangkok, Thailand, was characterized to determine their susceptibility to antibiotics. The genomes of 24 isolates displaying distinct levels of resistance were sequenced using the Illumina MiSeq platform and those of 3 strains exhibiting the highest resistance levels were additionally determined with the long-reads PacBio technology. Assembly of the PacBio-sequenced genomes allowed the identification of homologous megaplasmids in two out of the three selected isolates. Further characterization of the megaplasmids sequences revealed the presence of multiple genes encoding resistance determinants against a variety of antibiotics groups. Remarkably, several of these genes were linked to integrons in the two plasmids. Additional comparative analyses unveiled the presence of closely-related megaplasmids in other two multidrug-resistant Thai isolates and in non-aeruginosa Pseudomonas strains from databases.

Altogether, our findings highlight the commonly overlooked role that plasmids and other Mobile Genetic Elements play jointly on spreading antibiotic resistance in P. aeruginosa.
Title: Longitudinal Analysis of Enteric Pathogens Exposure Events in Healthy Malawian Children

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Abstract

Background: Children in developing countries like Malawi are exposed to risk factors that predispose them to enteric pathogenic organisms causing diarrhea. Diarrhea is among the leading causes of morbidity and mortality among children in SSA.

Method: Sixty healthy children were recruited at 6 months of age and followed up every month for 12 months, to the age of 18 months. Stool samples were collected and then tested using Enteric TaqMan array card assay, that detects up to 29 enteric pathogens. Pathogen exposure events at different time points, and their relation to participant age and clinical features were analysed.

Results: A total of 1526 positive targets were detected from 442 samples tested from all time points. E. coli and Enterovirus were detected in all participants at more than 75% of the time points. There was a very high rate of multiple pathogen detection per sample with 1.6%, 10.7% and 87% of samples having 0, 1 and >2 positive detection. Longitudinal analysis shows continuous exposure to E. coli and Enterovirus with no significant difference at different time points. Campylobacter and EPEC were significantly associated with diarrhea. A high rate of inter and intra individual variability was observed in pathogen detection at different time points.

Conclusions and further work: Malawian children are exposed to multiple pathogens very early in life. E. coli, which may have adapted to the gut microbiota of Malawian children, may not be clinically significant but needs to be further studied for the immununological effects it may exert in vaccine response, nutrition, and also its role in relation to other symptomatic enteric pathogen infections. Pathogen exposure will now be analysed longitudinally in relation to 16S microbiome profile among these children.
Title: Emergence of Pseudomonas aeruginosa small colony variants (SCVs) under environmental stress

Authors: H. Chowdhury¹, C. Winstanley¹, A. Kadioglu¹, D.R. Neill¹, J.L. Fothergill¹

1. Department of Clinical Infection, Microbiology and Immunology

Abstract

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium and is the major proven pathogen in patients with cystic fibrosis (CF) lung infections. P. aeruginosa undergoes extensive adaptation within the lung, in some cases forming a small number of phenotypically distinct morphotypes, called small colony variants (SCVs). Lung-adapted SCVs have been associated with low bacterial fitness, enhanced biofilm formation and increased resistance to antibiotics. However, the molecular basis of the evolution of SCV phenotypes in the CF lung environment is poorly described, as the genetic pathways associated with phenotypic conversion are often complicated by reversion to the normal phenotype. In this study, we have investigated the phenotypic and genetic basis of the evolution of SCVs isolated from a successful epidemic clone – Liverpool Epidemic Strain (LESB65) – harbouring a mis-sense mutation at the pmrB locus. Our initial phenotypic assays revealed an increased fitness and enhanced biofilm production under growth-limiting conditions for all the SCVs, features which may be hallmarks for persistence of infection in the lung. Using whole genome sequencing, we have identified parallel mutations in a number of loci, including mutation in 16S rRNA in two of the SCV isolates. Genome rearrangements encompassing rRNA operons have recently been reported to be associated with SCV phenotypes. Overall, our initial data provide an insight into the clinical relevance of SCVs during chronic lung infections.
Title: Developing an in vitro biofilm model for Streptococcus pneumoniae.

Authors: Emma Dearing¹, Daniel Neill¹, Aras Kadioglu¹

1. Institute of Infection and Global Health, Department of Clinical Infection, microbiology and Immunology.

Abstract

Research questions: Do pneumococci actually form biofilm (the definitive evidence is lacking) and if they do, are there serotypes differences? Does biofilm formation relate to virulence?

It is not universally accepted in the field of pneumococcal research that biofilm assays developed thus far provide accurate and representative models for biofilm formation by Streptococcus pneumoniae in vivo.

Here we describe investigations into alteration in pneumococcal phenotype using a fairly recently established biofilm model, in which bacteria are first exposed to fixed human airway epithelial cells in a low nutrient media. This allows for formation of biofilms before transfer onto live epithelial cells in regular, high nutrient tissue culture media. In other in vitro assays, when live epithelial cells are infected with a dose of planktonic S. pneumoniae, bacteria will proliferate and eventually cause epithelial cell death. However, when biofilms are formed in contact with fixed epithelial cells, bacteria do not proliferate at a high rate or kill live epithelial cells following transfer.

Initial results, using a wild type strain of S. pneumoniae, will include CFUs for biomass of biofilm through formation, antibiotic resistance in biofilms and looking at changes in capsule thickness during biofilm formation. Different serotypes will then be compared on the biofilm model to look for possible links between biofilm forming ability, adherence to epithelial cells and overall virulence.
Title: The Impact of Hormones on Pseudomonas aeruginosa Proteome

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1. Institute of Infection & Global Health, University of Liverpool
2. Institute of Translational Medicine, University of Liverpool

Abstract

Background: Urinary tract infections (UTIs) cause high morbidity, mortality and economic burden. Pseudomonas aeruginosa causes persistent UTI infections because it forms biofilms and can be resistant to multiple antibiotics. Previous studies indicate that sex hormones such as estradiol may modulate biofilm formation and dysregulate innate immune responses in females. Hence, this study aims to observe whether differences exist between varying types of growth media and to understand the role of hormones in UTI pathophysiology.

Methods: To investigate the proteomic profile of P. aeruginosa UTI clinical isolates, 5 ml of overnight culture was added to each of LB, Artificial Urine medium (AUM) and urine. Upon reaching the optical density of 0.25, the cultures were centrifuged at 4200 rpm and washed with PBS. Proteomic analysis was then performed.

Results: Assessment of proteomics data revealed fluctuating levels and abundance of virulence associated proteins produced in response to the medium. Siderophores such as pyoverdine and pyochelin displayed significantly higher levels of abundance in AUM when compared to LB. However, presence of sex hormones estradiol, testosterone and progesterone appeared to decrease protein expression in AUM. The same effect was observed in urine medium.

Conclusion: Analysis of proteomic data revealed different adaptation strategies implemented by P. aeruginosa and significant hormone-dependent alteration in the proteomic activity, particularly the reduction of siderophores. This may suggest that patients may have variable susceptibility to persistent infections based on their hormonal profile. Therefore, further research is required to understand the role of sex hormones in pathogen-host interactions.
Title: AZTEC-CF: Aztreonam lysine for inhalation in the treatment of pulmonary exacerbations of cystic fibrosis. Interim results

Authors: Freddy Frost¹, Dilip Nazareth², Jo Fothergill¹, Martin Walshaw², Craig Winstanley¹

1. Institute of Infection & Global Health
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Abstract

Introduction: Standard care for acute pulmonary exacerbations (PEx) of cystic fibrosis (CF) patients is currently treatment with two intravenous antibiotics, yet inhaled antibiotics may represent a better choice since they achieve much higher concentrations at the site of action with less systemic exposure.

Objectives: To investigate the clinical and microbiological effects of treating PEx with aztreonam lysine for inhalation (AZLI).

Methods: AZTEC-CF is an open-label cross-over study including 16 subjects with Pseudomonas aeruginosa infection. Over the course of consecutive hospitalisations patients received two treatments (Treatment A: AZLI plus intravenous colistimethate. Treatment B: Standard PEx care). Primary outcome is change in lung function (forced expiratory volume in 1 second [FEV1]) at 14 days. Secondary outcomes include time to next exacerbation, quality of life measures and sputum microbiology (colony forming unit (CFU) counts and 16S rRNA sequencing).

Interim Results: At interim analysis 10 study subjects had completed both arms of the study. At day 14 AZLI was associated with greater improvement in FEV1 (+13.5% vs. 11.5%, p=0.004). AZLI was also associated with longer time to next exacerbation (119 vs. 64 days, p=0.04). Early microbiological analysis suggests there was no difference in changes in total viable bacteria counts between treatment group but the AZLI arm was associated with a numerically greater reduction in P. aeruginosa counts (-2.0 vs. +1.04 logCFU/ml).

Conclusion: Interim analysis suggests inhaled antibiotics may represent a viable treatment for PEx in CF. Further analyses are underway to understand the impact of inhaled antibiotics the bacterial community in the CF lung.
Title: Identification of niche-specific virulence factors via experimental evolution of Streptococcus pneumoniae

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Abstract

Streptococcus pneumoniae (the pneumococcus) is an important human pathogen, adept at colonising various ecological niches within the host. Colonisation of the nasopharynx, followed by asymptomatic carriage and non-inflammatory clearance is the predominant outcome of infection, but diverse disease manifestations including pneumonia, septicaemia and meningitis occur in a minority of individuals.

Through experimental evolution of pneumococci in mouse disease models, we are investigating the genetic basis of the niche adaptations that enable pneumococci to switch from a commensal lifestyle in the nasopharynx, to a pathogenic phenotype in the lungs, brain or blood. Experimental evolution has been carried out via serial passage of pneumococci separately through pneumonia and nasopharyngeal carriage mouse models, to generate lineages adapted to the lung and nasopharyngeal environments, respectively. Starting from a non-passaged (lab adapted) isolate, ten independently-evolved lineages of lung-adapted pneumococci have been generated, each having been passaged 20-times through a mouse pneumonia model. Pneumococci recovered from the infected lungs were used to inoculate further mice for the next passage round.

We will present the results from phenotypic analysis of lung-passaged bacterial isolates including growth characteristics, toxin production, adherence to epithelial cells and antimicrobial resistance profiles. We will also describe how the adaptations acquired by pneumococci, which facilitate survival in the lung environment, can influence bacterial gene expression during exponential growth. These studies will provide insight into genetic changes associated with pneumococcal commensal to pathogen switch. Identifying such genetic determinants of virulence will be valuable for the development of vaccine candidates and targets for therapeutic intervention.
Title: Characterising models of lung injury using the biological toxin ricin, for subsequent use in the screening of novel candidate therapies for treating intoxication.

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Abstract

The recent conflicts worldwide have illustrated the willingness of both nation states and non-governmental groups to use weapons that are forbidden under the 1997 Chemical Weapons Convention. One such compound that is of particular concern is ricin. Ricin is a protein found within the seeds of the Ricinus communis plant. Ricin is commonly formed as a by-product of castor oil production and is considered a bioterror threat due to the low lethal dose limit and wide availability of raw material. Whilst ricin is toxic when delivered by ingestion or subcutaneous injection, it is delivery via inhalation that is particularly challenging from a clinical perspective. Currently, there are no specific medical countermeasures for the treatment of ricin intoxication and poisoning is difficult to evidence as the compound is rapidly degraded in the human body.

As part of a systems toxicology approach, the effects of ricin exposure on the pulmonary proteome has been investigated. Firstly, label free quantitative proteomics was used to study the temporal effect of ricin delivered by the aerosolised route on the mouse lung. Bioinformatics analysis was employed to form a relative picture of biological effect. Comparative transcriptomic analysis and DCQ of ricin exposed murine lung tissue, has been used to complement the investigation. We have demonstrated that pathways involved in the inflammatory and innate immune responses follow a post exposure trajectory whereas, pathways and proteins involved with cell adhesion and redox processes decreased following exposure. Understanding the composition and complexity of the unique biological response will be essential for successful development of medical countermeasures. Further work aims to verify the approach using direct MinION RNA sequencing and development of improved cell culture models.

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Title: Study of Mosquito Vectors in Relation to Avian Malaria and Host Preferences in UK Zoos

Authors: Arturo Hernandez-Colina\textsuperscript{1,2}, Merit Gonzalez-Olvera\textsuperscript{1,3}, Emily Lomax\textsuperscript{1,2}, Matthew Baylis\textsuperscript{1,2}

1. IGH
2. EPH
3. IB

Abstract

Vector-borne diseases have become a major threat for the health of human populations and endangered wildlife. For instance, avian malaria is caused by the parasite Plasmodium spp. and can cause serious mortalities in captive penguins and even the extinction of endemic birds.

We collected mosquitoes during 2017 and 2018 in two UK zoos and identified them by morphology and molecular techniques. To understand the mosquitoes host preferences, we analysed the abdomens of the ones with fresh blood doing a nested-PCR and comparing the sequences in databases. We screened the mosquitoes with another nested-PCR for the avian malaria parasite.

We collected over nine thousand mosquitoes in 2017; the majority were Cx. pipiens (95%), the main avian malaria vector. We analysed 288 blood meals and identified the host in 94, including humans, birds and zoo animals; we observed mixed-bloods of humans and birds. Comparing the locations of the zoo animals and the traps, we estimated the mosquitoes flying distances. The avian malaria parasite prevalence was around 10% and the main species found was P. matutinum.

Our results show differences in the mosquitoes’ abundance and feeding activity by areas and months; therefore, particular considerations should be taken. The mixed-blood feeding behaviour alerts us of transmission risks as birds are reservoirs of zoonotic pathogens like West Nile virus. The analyses of this year’s samples still ongoing, but our results have been already applied to the control of the mosquito population and prevention of the infection in the study sites.
Title: Can *Fasciola hepatica* metacercariae survive ensiling and retain their viability?

Authors: Bethan John¹, Jane Hodgkinson¹, Diana Williams¹, David R Davies²

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2. Silage Solutions Ltd, Bwlch y Blaen, Pontrhydygroes, Ceredigion, SY25 6DP, United Kingdom

Abstract

Fasciola hepatica, the common parasitic liver fluke, is an important cause of morbidity and mortality in ruminant livestock worldwide. Effects of sub-clinical infection on growth rate and milk yield is estimated to cost the UK cattle industry up to £40.4 million annually. The liver fluke life cycle is complex, due to free-living parasite stages and its dependency on the presence of an intermediate host, a mud snail, Galba truncatula. It is understood that up to 50% of metacercariae can overwinter on pasture and infect grazing livestock the following spring. However, the risk of infection posed by feeding silage to livestock is not well understood. The majority of studies investigating metacercarial viability following ensiling are outdated and do not implement molecular techniques to detect the persistence of *F. hepatica* DNA within silage. Moreover, the impacts of aerobic spoilage on metacercariae survival and the potential development of ensiled metacercariae into infective newly excysted juveniles (NEJs) must be better understood in order to determine the importance of silage in disease transmission.
Title: Helminth co-infection increases pneumococcal carriage density and dissemination to lungs and blood

Authors: Alice E. Law1, Rebecca Shears1, Richard K. Grencis2, Aras Kadiglu1, Daniel R. Neill1

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Abstract

Background: Nasopharyngeal colonisation by Streptococcus pneumoniae (the pneumococcus) and chronic gastrointestinal infection with soil-transmitted helminths (STHs) are common events of childhood in many lower and middle income countries (LMICs). It is likely that a large proportion of children worldwide are concurrently-infected with the pneumococcus and one or more helminths.

The ability of helminths to modulate systemic host immunity to ensure chronicity is well established, with polarisation towards a Th1 or T regulatory cell-dominated response often playing a crucial role. Given the importance of balance between these pathways in maintaining asymptomatic pneumococcal carriage, we aimed to determine if STH-driven immune perturbation would have a significant impact on carriage and invasive pneumococcal disease (IPD) susceptibility.

Methods: We investigated the effects of a strictly enteric helminth infection with Trichuris muris on S. pneumoniae nasopharyngeal carriage, using a novel murine model.

Results: T. muris infection led to reduced host control of pneumococcal carriage with increased bacterial loads in nasopharynx and enhanced dissemination into the lungs and blood. An early inflammatory phenotype dominated in the nasopharynx and lungs of co-infected mice, characterised by neutrophil infiltration, classically activated macrophages and increased production of pro-inflammatory cytokines. Importantly, we highlight the potential of anthelminthic treatment to lessen this inflammatory phenotype, reducing bacterial loads and promoting clearance from the nasopharynx.

Conclusions: These data address a previously unrealised health issue that may have major impact on public health systems in LMICs, providing insight into pneumococcal dynamics and potentially informing treatment options to combat IPD where Trichuris infections are common.
Title: The cost of diagnostic uncertainty: A prospective economic analysis of 8,552 febrile children attending an 
NHS Emergency Department

Authors: S Leigh¹, A Grant², N Murray³, B Faragher⁴, H Desai⁵, S Dolan⁶, N Cabdi⁷, J Murray⁸, Y Rejaei⁹, S 
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Abstract

Background: Paediatric fever is a common cause of emergency department (ED) attendance, yet a lack of 
prompt and definitive diagnostics makes it difficult to distinguish viral from potentially life-threatening 
bacterial causes. This may result in extended periods of observation, radiography, and the precautionary use 
of antibiotics (ABs) prior to evidence of bacterial foci.

Methods: We studied a prospective-representative cohort of 8,552 febrile children attending Alder Hey 
Children’s Hospital over a one-year period. Using a time-driven and activity-based micro-costing approach we 
estimated the economic impact of managing paediatric febrile illness, identifying patient and healthcare 
provider characteristics associated with both increased resource use, and potentially avoidable AB prescribing.

Results: Febrile infants (<3 months) incurred the greatest resource use at £1000.28 [95%CI £746.56-
£1,253.99]) per child; with lesser experienced doctors also incurring 3.2-fold [95%CI 2.0-5.1-fold] higher 
resource use than consultants, (p<0.001). Approximately 32.4% of children received antibiotics, with 7.1% 
retrospectively confirmed with bacterial infections. Children with viral illnesses for whom antibiotic use was 
potentially avoidable incurred 8.6-fold [95% CI 5.95-12.01-fold] cost increases; corresponding to an additional 
£1,491.02 per child. Approximately £1-in-every-£6-spent in the ED resulted from potentially avoidable 
antibiotic prescribing.
Conclusions: The economic impact of uncertainty when managing paediatric fever is significant, and the precautionary use of antibiotics increases costs significantly. The use of ED resources is highest among infants and children managed by lesser experienced doctors. Diagnostic advances which may increase confidence to withhold antibiotics, would yield substantial efficiency gains in these groups; where the perceived risks of failing to identify potentially life-threatening bacterial infections are greatest.
Title: Evaluation of Human T and B Cell Immune Responses in Nasopharynx-Associated Lymphoid Tissue (NALT) to Live Attenuated Oral Vaccine Against Rotavirus

Authors: León-Rios, Miguel1, Cunliffe, Nigel1, Iturriza-Gomara, Miren1, Zhang, Qibo1

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Abstract

Background: Mucosal vaccines have been recognized as an effective way of immunization against enteric pathogens. These vaccines have shown to stimulate a local production of antigen-specific antibodies and the development of long-term B and T memory cells promoting both a mucosal and systemic protective immune response. Despite the effectiveness of the vaccine in reducing disease burden in general, there are significant differences in vaccine efficacy between developed and developing countries, although the immunological mechanisms remain undefined. Here we evaluate the immunogenicity of live attenuated oral rotavirus vaccine using an ex vivo cell culture model with tonsillar tissues from children and adults, a key mucosal compartment mediating mucosal immunity in humans, and examine the effect of pre-existing immunity on vaccine-induced mucosal immune response.

Methods: 50 immunocompetent patients referred to adenotonsillectomy were included in this study. Serum samples were collected from these patients and rotavirus-specific antibodies were measured by ELISA. Mononuclear cells from tonsil tissues of children (n=7) and adults (n=5) were isolated and stimulated with Rotarix vaccine. T cell proliferation levels and antibody response in cell culture supernatants were measured by flow cytometry and ELISA respectively.

Results: Rotavirus-specific IgG levels were detected in serum from all recruited patients. A positive correlation was observed between serum rotavirus-specific IgG titres and patients age. Rotavirus-specific IgG levels were also detected in tonsillar cell supernatants following Rotarix vaccine stimulation. There was no significant difference in the mucosal B cell antibody production between children and adults. Pilot data indicate the vaccine stimulation elicit a CD4+ and CD8+ T cell proliferative response in children. Studies are ongoing to analyse the effect of pre-existing immunity on the vaccine-induced mucosal T cell and B cell responses in children.
Title: Hazara Virus as a Model for ‘Omics Analysis of Crimean-Congo Hemorrhagic Fever Virus Infection in Tick Cell Lines

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Abstract

Ticks are vectors and/or reservoirs of many highly-pathogenic arboviruses affecting humans and livestock, some of which occur in, or threaten, the UK. Crimean-Congo hemorrhagic fever virus (CCHFV) causes a major emerging zoonotic tick-borne disease with a reported human case fatality rate of up to 40%, while infected ruminant hosts show no clinical signs. The geographical reach of CCHFV is limited by the distribution of its primary vectors, ticks of the genus Hyalomma. However, recent climate change and increased global trade have led to expansion of the range of many tick species and the pathogens that they transmit, and international travel increases the risk of exposure of UK residents to tick-borne viruses, exemplified by CCHF cases in 2012 and 2014. Tick cell lines can be used to elucidate virus-vector interactions in vitro. Studying tick-virus interactions for CCHFV is challenging due to the need to work at biosafety level (BSL) 4; however, exploiting the closely-related but apathogenic Hazara virus (HAZV) in tick cell lines provides a suitable model system in a BSL2 setting. Here we examine the growth kinetics of HAZV in cell lines derived from two Ixodes spp. (the natural host genus of HAZV) and Hyalomma anatolicum (a vector of CCHFV), using quantitative RT-PCR to measure viral RNA replication and focus-forming assay in mammalian cells to measure virus production. Our results will enable us to select suitable time points for development of protocols for -omics analysis of virus-vector cell interactions that will subsequently be applied to CCHFV.
Title: Modulation of neutrophil migration by the pneumococcal toxin, pneumolysin

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Abstract

Background: Pneumolysin (PLY), is a membrane-damaging toxin synthesized by Streptococcus pneumoniae, which plays a multifunctional role as a pneumococcal virulence factor during infection. It has various biological activities, with distinct cytolytic and sub-lytic effects that interfere with cell function. The interaction of PLY with neutrophils is very complex and it remains unclear if it inhibits or enhances neutrophil migration when acting directly on these cells. It has been suggested this toxin can directly modulate neutrophil migration. However, the precise effects on neutrophil motility and the molecular mechanism underpinning these changes remain unknown.

Method and Results: We demonstrate that PLY inhibits neutrophil chemotaxis towards KC, but does not competitively act as a chemoattractant for neutrophils. Consistent with this reduction in migration, we also show that PLY treated neutrophils have strong adhesion to tissue culture plastic, and display enhanced actin polymerization. Quantitative label-free proteomics of PLY-treated neutrophils revealed four proteins related to cell migration that significantly changed in abundance in PLY-treated neutrophils: Tyrosine-protein kinase Fes/Fps (FES), Grancalcin (GCA), Tyrosine-protein kinase CSK (CSK) and FYVE, RhoGEF and PH domain-containing protein 3 (FGD3). These offer potential candidates to better understand the inhibitory effect of PLY on neutrophil chemotaxis.

Conclusion: The current report provides new insight that may be a useful tool in understanding how PLY alters neutrophil migration in S.pneumoniae infection. Understanding how neutrophils migrate through inflamed tissues is important in order to develop therapies for controlling their activity.
Title: Immune-complex mimetics (ICMs): adjuvant free approach to vaccination and improved immune diagnostics for the control of flaviviruses.

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Abstract

Many important flavivirus diseases have no vaccine available. Zika virus caused hundreds of thousands of infections in 2015/16, as well as many thousands of cases of congenital Zika syndrome (CZS). Many vaccines are under development, but these require co-administration with adjuvants that are difficult to manufacture, are often unstable, and can have undesirable side effects. The use of adjuvants also greatly adds to the overall cost of vaccine development in low income settings.

Multimeric Fc-fusion proteins mimic immune-complexes by delivering antigens directly to antigen presenting cells, therefore ensuring that antigen processing is efficient as possible. This may allow delivery of effective vaccines that do not rely on adjuvants. The multimeric structure of the constructs allows for cross-linking and triggering of critical Fc-receptors that is not possible with monomeric Fc-antigen fusions or protein-in-adjuvant approaches.

We are developing multimeric Fc-fusion proteins containing the envelope protein domain III (EDIII) epitope from Zika virus, as well as from other major flaviviruses. EDIII have been cloned into a highly engineered Fc-fusion expression plasmid and the vaccine produced by expression in CHO-K1 cells. We demonstrate that the vaccine is recognised by convalescent sera from flavivirus-infected patients and can engage relevant human and mouse receptors required for optimal immune responses. By measuring expression of key maturation markers, we have established that the vaccine can drive maturation of human dendritic cells, an important requirement for a successful vaccine. Next, we will further investigate immunogenicity of the vaccine by testing efficacy of the constructs in vivo. The outcome of this work is to develop novel vaccines, not only against Zika, but also other flaviviruses.
Title: The role of calmodulin on Ebola virus transcription and replication and its potential as an antiviral target.

Authors: Jordana Muñoz-Basagoiti¹, Isabel García-Dorival¹, Stuart D Armstrong¹, Miles W Carroll², Julian A Hiscox¹

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Abstract

Ebola virus disease (EVD) is a deadly disease in humans characterized by severe immunosuppression, high virus replication and a case-fatality rate of up to 90%. Efficient vaccines and therapeutics to fight EVD are currently unavailable. Viral genomes require the host cell machinery for genome replication, transcription and translation. The viral riobonucleoprotein (RNP) complex is viral genomic RNA associated with four viral proteins, which are the RNA-dependent RNA polymerase (L), VP35, VP30 and the nucleoprotein (NP). This complex constitutes the minimal essential unit for EBOV genome transcription and replication. These processes involve virus-host protein-protein interactions (PPIs) and their use as drug-targets are an attractive antiviral therapeutic alternative.

In this study, a recombinant EBOV L protein with a fluorescent protein mCherry inserted in a non-functional region of it was designed. Rabbit reticulocytes permitted high amounts of recombinant protein expression. L-mCherry was co-immunoprecipitated and label-free proteomics allowed the elucidation of virus-cell PPIs. Calmodulin (CALM) was identified as an L-host interactor. Functional assays with the small molecule inhibitor W-7, antagonist of CALM, were assessed. For that, an EBOV minigenome system, which mimics viral transcription and replication, allowed the study of the inhibition of CALM on the EBOV RNA synthesis under biosecurity level 2 (BSL-2) facilities. Indeed, its inhibition had a detrimental effect on the reporter activity of the system, suggesting that the association between L and CALM may be essential for viral RNA synthesis and that CALM might have potential use as an antiviral therapeutic for EVD.
Title: First detection of Orthohepevirus C (rat Hepatitis E virus) in a brown rat from the United Kingdom and the potential implications for public health

Authors: Ellen Murphy¹, Nicola Williams¹, Lorraine McElhinney², Daisy Jennings², Julian Chantry¹, Ranieri Verin¹, Malcolm Bennett³

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Abstract

In the United Kingdom, there has been a steady annual increase in the number of Hepatitis E virus (HEV) infections in people since 2010. Most of these are thought to be indigenously acquired Orthohepevirus A genotype 3 (HEV G3) which has been linked to pork production and consumption. However, the dominant subgroup circulating in pigs differs from that which is found in people, therefore an alternative, potentially zoonotic, source is suspected as a possible cause of these infections. Rodents, brown rats (Rattus norvegicus) in particular, have been shown to carry HEV, both the swine HEV G3 and Orthohepevirus C, genotype C1 (rat HEV). To investigate the prevalence of HEV in British rodents liver tissue was taken from 307 rodents collected from pig farms and other locations. The RNA from these samples was extracted and screened using a pan-HEV nested RT-PCR. Histopathology was also performed. In this study 8/61 (13%, 95% CI, 5-21) of brown rat livers were positive for HEV RNA. All of the positives were found to be rat HEV with 89-92% nucleotide identity to other rat HEV sequences circulating within Europe. Lesions and necrosis was observed histologically in 2/3 samples examined. No HEV RNA or HEV G3 (Swine or human variant) was detected in any rodent species in this study. This is the first reported detection of rat HEV in a wild rat from the United Kingdom. Further study are required to assess the zoonotic potential and risk to public health.
Title: *Pseudomonas aeruginosa* as an intracellular pathogen in urinary tract infections

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3. Dept of Clinical Infection, Microbiology and Immunology, University of Liverpool

Abstract

Urinary tract infections (UTIs) are the most common healthcare acquired infections and are associated with substantial economic and human cost. *Escherichia coli* is the primary causative agent of UTIs, however a small yet significant portion of UTIs are caused by *Pseudomonas aeruginosa*. *P. aeruginosa* has been identified as a pathogen of the highest concern in the global antibiotic resistance crisis. *P. aeruginosa* is frequently studied in the lung infections of cystic fibrosis patients where it has been shown capable of invading human epithelial cells. Bacterial invasion of host cells is considered to be a valuable mechanism for evading the host immune system. Several pathogens associated with UTIs have been shown to invade host cells of the urinary tract but there is no literature on the potential invasiveness of *P. aeruginosa* in the urinary tract. We attempted to elucidate whether or not several clinical isolates and lab strains of *P. aeruginosa* were capable of invading bladder urothelial cells in an in vitro model. Gentamicin protection and adherence assays were carried out on immortalized bladder urothelial cells. We saw variability in levels of invasiveness across several clinical isolates and lab strains of *P. aeruginosa*. Further work is needed to discover how and why the studied strains varied in their invasiveness and whether the results translate to more physiologically relevant models of the human urinary tract.
Title: Determining the rate evolution of Ebola virus under different cellular challenges.

Authors: Rebekah Penrice-Randal¹, Lisa Ng², Julian Hiscox¹

1. University of Liverpool
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Abstract

Ebola Virus is the aetiological agent for an acute haemorrhagic fever in humans and non-human primates. Zaire ebolavirus was responsible for the devastating outbreak in West Africa 2013-2016, where out of approximately 28,652 suspected cases there were 11,325 deaths following zoonotic transmission from bats. Ebola virus is a 19kb non-segmented negative sense single stranded RNA virus. RNA viruses are susceptible to the incorporation of mutations into the viral genome during replication facilitated by the RNA-dependent RNA polymerase (RdRp). Thus, permitting rapid adaptation to selection pressures such as medical countermeasures and evolution of the viral genome. Whilst the rate of evolution of the Ebola virus genome was established in the recent outbreak by work undertaken in our laboratory, the actual error rate of viral replication under different pressures has not been defined. Particularly in terms of potential innate intrinsic anti-viral pathways and physiological responses to infection. The aim of this project is to study error rate using both sub-viral systems and infectious virus in biologically relevant conditions. Sub-viral systems, such as minigenomes provide the opportunity to study the replication and transcription of category level 4 viruses at biosafety level 2 containment. The guanosine analogue, ribavirin, was added to cells transfected with the minigenome system to determine whether mutations can be detected using RNAseq and the newly emerging field of MinION sequencing. Additional most of the experiments performed in cell culture do not reflect physiological conditions found inside the body so the behaviour of the EBOV RdRp was investigated under anoxic conditions focusing on HIF-1A, a cellular protein stimulated during the innate response. Such comparisons allow insight of the error rate of the RdRp and the influence of host biology to drive viral evolution.
Title: Validating diagnostic markers that may predict the outcome of Ebola virus patients

Authors: Jocelyn Pérez¹, Neil Blake¹, Lisa Ng², Julian Hiscox¹

1. Institute of Infection and Global Health
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Abstract

Background: Ebola virus (EBV) is one of the deadliest re-emerging viruses causing haemorrhagic fever. The viral load measured as Ct value by Quantitative reverse transcription PCR (RT-qPCR) is currently the gold standard to predict the outcome of EBV patients. Triage is appropriate when Ct values are high or low but not in mid-range CT values. Therefore, we aim to develop a multiplex RT-qPCR based on 10 host biomarkers identified by transcriptomic analysis, that showed a higher predictive value than viral load.

Methods: The genes selected as potential markers to predict the Ebola virus disease patient’s outcome are: VCAM1, HOPX, TUBG1, PLPP3, MS4A4A, TGFBI, TTC28, NIF3L1, SLC25A5, and CTSL. In order to develop a singleplex RT-qPCR assay for the detection of each gene, in-vitro RNA standards were generated. These standards were used for the optimization of each one-step RT-qPCR assay using SYBR Green.

Results: Primer sets for CTSL, NIF3L1, SLC25A5, VCAM1, TGFBI, TTC28, showed a high PCR efficiency (> 90%) and a correlation coefficient greater than 0.99. The optimized RT-qPCR assays were evaluated using blood samples from 5 healthy donors. As a pilot study, clinical samples from 9 survivors and 9 fatal cases are being evaluated for VCAM1.

Conclusions: To date, primers for 6 genes showed a high PCR efficiency. RT-qPCR assays for HOPX, TUBG1, MS4A4A and PLPP3 need to be further optimized. Future work will focus on validation of all primer pairs, and screening blood samples from Ebola-infected patients. Longer term the goal is to develop a one-step multiplex RT-qPCR for a gene set that can be used as in-field diagnostic tool for EVD.
Title: The Tropism and Transstadial Transmission of a Rickettsia endosymbiont in the Highland Midge

Authors: Jack Pilgrim¹, Gregory Hurst², Stefanos Siozios², Matthew Baylis¹

1. Institute of Infection and Global Health
2. Institute of Integrative Biology

Abstract

Biting midges of the genus Culicoides are the vectors of several veterinary pathogens. Current disease control relies primarily on vaccines which, given the emergence of new serotypes, are often unavailable. Thus, the developments of novel control interventions are of pressing relevance. Recent research has demonstrated inherited bacteria (endosymbionts) can alter the vector competence of their insect host representing a viable means to control vector-borne diseases. Our previous work has shown the endosymbiont Rickettsia (group Limoniae) to be widespread in midge populations with frequencies often reaching fixation. Through Fluorescence in-situ hybridisation (FISH) screening, this study focuses on the unique Rickettsia tissue tropisms observed in the highland midge (Culicoides impunctatus). Of particular interest are infections detected in the ovaries, ovarian suspensory ligament and spermathecae, as well as in the heads of larvae. This combination of germline and somatic tissue infections provide insights into the transmission strategies of Rickettsia, as well as their potential to alter the capacity of their host to transmit arboviruses.
Title: The role of SIGLEC1 in Japanese Encephalitis

Authors: Tessa Prince1, Raquel Medialdea Carrera 2, Lucille Rainbow2, Tom Solomon1, Neil Blake1, Mike Griffiths1

1. IGH
2. IIB

Abstract

Japanese Encephalitis Virus (JEV) is a major cause of viral encephalitis in Asia and has spread to regions of the world where it has previously been unseen. Whole blood transcriptomics on the blood of Nepalese children with acute encephalitis syndrome (AES) has found up-regulation of several transcripts involved in the type I interferon response in patients diagnosed with Japanese encephalitis, in contrast to those with an unknown aetiology. Once such transcript was SIGLEC1. Little is known on the role of SIGLEC1 during flavivirus infection, therefore this study aims to examine the role of SIGLEC1 in Japanese encephalitis.

Small interfering RNAs (siRNA) were used to knockdown expression of SIGLEC1 and RSAD2 in human transformed microglia. Wild type and knockdown microglia were infected with JEV at an MOI of 10 for 24 hours and plaque assays and JEV qPCR used in order to determine what effect SIGLEC1 knockdown had on viral load in cell supernatants.

SIGLEC1 knockdown resulted in significantly increased viral release from knockdown microglia compared to wild-type microglia (p=0.015). RSAD2 knockdown also resulted in increased viral load in the supernatant of knockdown microglia compared to wild-type microglia (p>0.05). While addition of JEV caused an increase in RSAD2 expression in wild-type microglia (p=0.008), addition of JEV did not cause any change in the expression of SIGLEC1 in microglia (p
Title: Extracellular Histones Enhance Phagocytosis

Authors: Yasir Qaddoori, Simon T Abrams, Jesús Reiné, Ben Morton, Guozheng Wang and Cheng-Hock Toh

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Abstract

Background: Histones are released extracellularly following extensive cellular damage or infection, and are elevated in the circulation of septic patients. As well as being toxic, circulating histones form part of the innate immune response, acting as damage-associate molecular patterns (DAMPs) and inactivating complement. DAMPs can activate pattern recognition receptors, such as Toll-like receptors (TLRs) on phagocytes, to facilitate bacterial clearance. However, the specific pathophysiological role of extracellular histones in this process remains unknown.

Methods: In vitro phagocytosis of GFP-E.coli uptake using a PMA-activated U937 cell line was quantified by fluorescent microscopy and Western blot. Mechanistic studies were performed using anti-TLR2/4 neutralizing antibodies. Ex vivo histone-induced phagocytosis was examined in whole blood from healthy donors spiked with histones, and in septic patients using an intra-phagosomal bead-based assay, quantified by flow cytometry.

Results: Histones significantly enhance phagocytosis of live GFP-tagged bacteria, by PMA-activated U937 cells in a dose dependent manner (0-20µg/ml). We found that blocking TLR4, but not TLR2 could partially inhibit histone-induced phagocytosis. Within the range of 0-20µg/ml histones (sub-toxic concentrations) we found that histones significantly enhanced the phagocytosis of intra-phagosomal beads by neutrophils in normal healthy blood, which reached a plateau at 20-50µg/ml histones (toxic concentrations). Using this assay in blood from septic patients, we found significant association between circulating histone levels and phagocytosis of intra-phagosomal beads.

Conclusion: Extracellular histones enhance phagocytosis, which may play an important role in the clearance of pathogens in septic patients. Furthermore, we highlight that histone-TLR4-myD88 pathway may play an important role in this process.
Title: Identification and functional characterisation of host proteins that interact with non-structural proteins of porcine reproductive and respiratory syndrome virus-1

Authors: S. Riccio1,2, B. Jackson1, J. Hiscox2, S. Graham1, J. Seago3

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Abstract

The porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of arguably the most important infectious disease affecting the global pig industry. PRRSV causes respiratory disease in piglets and reproductive failure in sows and consequently impacts both growing and breeding sectors. Both live attenuated and inactivated PRRSV vaccines are available and are widely used, however, they are failing to control the PRRS panzootic. PRRSV produces at least 16 non-structural proteins (NSPs) which are involved in viral replication and/or modulating the host immune responses. An improved understanding of virus-host interactions that lead to immunomodulation could aid the design of improved vaccines. Therefore, the aims of my project are to identify and characterise novel PRRSV-1 NSP-host protein interactions. NSP1α and NSP1β from PRRSV-1 subtype 1 strain 215-06 were screened using the yeast-2-hybrid (y2-h) system and a cDNA library generated from porcine alveolar macrophages - the virus’ primary target cell. The screen identified 62 and 127 putative binding partners for NSP1α and NSP1β, respectively. Potential binding partners involved in IFN signalling, the NF-κB pathway, ubiquitination and nuclear transport have been selected to confirm and characterise. Identifying and characterising novel interactions between PRRSV-1 NSPs and porcine proteins will increase our understanding of how the virus modulates the host cellular immune response. As current vaccines lack efficacy, new information discovered through this project could be exploited to rationally attenuate PRRSV as a basis for improved vaccines.
Title: Evaluation of mucosal immunity activated by an adenovirus vectored MERS-CoV vaccine in in Human Mucosal Nasopharynx-Associated Lymphoid Tissue of Children and Adults

Authors: KJ Shrwani¹, R Sharma², M Krishna², S Leong³, N Cunliffe¹, Q Zhang¹

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Abstract

Background: Middle East respiratory syndrome coronavirus (MERS-CoV) has recently emerged as a novel pathogen that causes severe respiratory tract disease. MERS-CoV infection has been reported in 27 countries worldwide, resulting in over 2000 laboratory-confirmed cases and 791 deaths. Consequently, developing an effective vaccine is in pressing need to protect populations against MERS-CoV.

Objective: We aimed to evaluate the immunogenicity of a novel adenovirus vectored MERS-CoV vaccine ChimpOx simian spike protein (ChAdOx1-MERS-CoV Spike protein) using an ex-vivo cell culture system modelling human nasopharynx-associated lymphoid tissue (NALT) from children and adults.

Methods: Adenotonsillar mononuclear cells (MNCs) were co-cultured with three concentrations (10³, 10⁴ and 10⁵) of the MERS-CoV vaccine followed by measurement of T cell proliferation by Carboxyfluorescin succinimidyl ester (CFSE) staining/flow-cytometry and of anti-MERS-spike protein body production using enzyme-linked immunosorbent assay (ELISA) assay.

Results: Our pilot results show that both CD4+ and CD8+ T cell proliferative responses to the vaccine stimulation were shown in MNCs from children and adults, although variations exist between individuals. Antibody production appeared to show in NALT tissue from some subjects, although not in others.

Discussion/conclusion: These findings suggest that stimulation with ChAdOx1-MERS-CoV vaccine leads to both CD4+ and CD8+ T cell response in human nasopharyngeal mucosal immune system. Pilot results also indicate it may activate anti-MERS-CoV antibody production. More studies are currently ongoing to investigate the immunogenicity of this novel vaccine including the cytokine production and functional T/B cell immunity.
Title: Correlative light and electron microscopy: Advanced technique to evaluate the ultrastructural distribution of LNA antisense oligonucleotides within the rabbit retina

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Abstract

Background: Locked nucleic acids-containing antisense oligonucleotides (LNA ASOs) are synthetic RNA sequences of interest for a variety of therapeutic indications including ocular conditions. ASOs can be applied via intravitreal injection to target retinal diseases. Although tissue distribution can be evaluated by immunohistochemistry, the complexity of the ocular structures requires more sophisticated approaches to assess LNA distribution at (sub-)cellular level. Objectives: To investigate the ultrastructural distribution of an LNA compound with correlative light and electron microscopy (CLEM), a novel techniques that combines immunofluorescence with transmission electron microscopy on the same tissue section.

Methods: Rabbit retinas were collected after intravitreal injection of ASO or PBS, processed, embedded in methacrylate resin and polymerized by UV light. Sections were placed on grids and stained by an antibody targeting phosphorothioate modifications of LNA compounds, and imaged with a fluorescence microscope. Next, the grids were contrasted and imaged with a transmission electron microscope. CLEM image was achieved by overlying fluorescence and ultrastructural images to assess ASO distribution.

Results: ASO accumulated in the retinal pigment epithelium, photoreceptor, Müller and ganglion cells, and macrophages. The antibody labelled variable vesicular structures, ranging from vesicles filled with electron-dense particles over multivesicular and multilamellar bodies to amorphous, moderately electron dense material.

Conclusion: This study demonstrates both the feasibility of CLEM to evaluate distribution of ASOs and the usefulness of CLEM in a preclinical research setting. The main advantage of the technique on ocular tissues is the possibility to locate the region of interest and subsequently examine it at very high magnification.
Title: Cross-reactive CD8 T-cell responses to Japanese encephalitis virus and dengue virus can recognize Zika virus sequences

Authors: Krishanthi Subramaniam¹, Shona Moore², Filippo Tatullo³, Raquel Medialdea Carrera³, Ayako Kurioka², Laura Scott¹, Daniela Weiskopf², Sutee Yoksan⁴, Paul Klenerman³, Tom Solomon¹, Lance Turtle¹

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Abstract

Flavivirus diseases such as dengue, yellow fever, Zika and Japanese encephalitis (JE) are associated with significant morbidity and mortality globally. Due to their similarity, the host immune response is cross-reactive, which can be protective or disease-enhancing. During dengue infection, CD8 T-cells may be protective or pathogenic whereas in Zika or JE they are likely protective. We examined whether CD8 T-cell responses against dengue virus (DENV) and/or Japanese encephalitis virus (JEV) could recognize Zika virus (ZIKV), and whether the response had anti-viral activity. Our study included three individuals with known responses against DENV and JEV that were recruited previously, and 26 newly recruited subjects. Seventeen of the 29 subjects (59%) had dengue exposure, two had recovered from JE, seven had received flavivirus vaccines (JE and/or yellow fever) and two were negative controls without known flavivirus exposure. PBMCs isolated from each subject were used to screen against JEV and DENV peptide libraries by IFN-γ ELISPOT. Of the 26 newly recruited subjects, 12 demonstrated positive IFN-γ ELISPOT results. We identified 9 minimal CD8 T-cell epitopes, 5 which cross-react with ZIKV variants in ex-vivo IFNγ assays and 4 with ZIKV sequence homology. Short-term T-cell cultures expanded to given DENV/JEV peptides showed potential to lyse cells labelled with ZIKV peptides, suggesting that CD8 T-cells primed to DENV or JEV could recognise and kill ZIKV-infected cells. These results reveal the importance of incorporating CD8 T-cell antigenic determinants in vaccine design for flaviviruses.
Title: *Mycobacterium tuberculosis* (Mtbc) derived liposomes from pathogenic and non-pathogenic strains differentially modulate HIV-1 infection

Authors: Jordan Thomas, Marion Pouget, Alessandra Ruggiero Georgios Pollakis, William A Paxton

1. Institute of Infection and Global Health, Department of Clinical Infection, Microbiology, and Immunology, University of Liverpool

Abstract

Background: Mtbc infection is the most common cause of mortality in HIV-1 infected individuals. We aimed to determine the effect different Mtbc derived glycolipids have on modulating HIV-1 infection by quantitating the variant life-cycle forms (Total, 2LTR and proviral DNA).

Materials and Methods: Liposomes containing glycolipids of pathogenic and non-pathogenic Mtbc were produced. CD4 T cells were infected with HIV-1 X4 or R5 strains in both cis- (direct infection) and trans- infection (capture/transfer with immature dendritic cells (iDC) in the presence of Mtbc liposomes. DNA was extracted from infected cells and HIV-1 DNA forms including total HIV DNA, 2-LTR circular DNA and integrated DNA were quantified via sensitive qPCR assays.

Results: For HIV-1 R5 cis-infection, Mtbc lipids from strains HN878 and CDC1551 were shown to significantly increase levels of HIV-1 2-LTR circular DNA (p=0.0378 and p=0.0022, respectively). For X4 HIV-1 cis-infection, strain EU127 and vaccine strain BCG were shown to significantly decrease levels of 2-LTR HIV DNA (p=0.00441 and p=0.0022, respectively). For HIV trans-infection, no significant difference was observed in HIV DNA quantifications, however, HN878 was shown to increase both R5 and X4 HIV replication. In iDC/CD4 co-cultures (cis and trans-infection), H37Rv liposomes were shown to increase whereas HN878 and CDC1551 were shown to decrease HIV-1 X4 replication. Conversely, co-cultures showed that HIV-1 R5 replication increased in the presence of HN878 and CDC1551 liposomes.

Conclusions: Our data show that Mycobacterium derived lipids have differential effects on HIV-1 replication and infection, and that these differences depend on HIV-1 tropism and cell-types.
Title: Lyme disease Surveillance in the United Kingdom 1998 – 2015

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Abstract

Background: The United Kingdom’s (UK) Lyme disease incidence figures aren’t currently reported at a national level. Figures are derived from two-tier confirmatory laboratory diagnostic results, compiled by two reference laboratories. Reporting national Lyme disease incidence based on laboratory confirmation has the potential to underestimate incidence, as clinical cases managed without diagnostics are missed.

Methods: This work assesses an array of UK datasets to describe the socio-demographic characteristics of Lyme disease patients in different health care settings. The three main datasets comprised of laboratory confirmed cases, hospital records, and primary care electronic health records.

Results: All three showed a classic bimodal age distribution, had significantly more cases in rural locations, showed an inverse linear relationship between the number of cases and societal deprivation, and displayed peak incidence in summer months. Primary care patients were significantly more like to be white, were equally likely to be of either sex, annual incidence significantly increased across the UK peaking in 2015 at 7.1 cases per 100,000. Hospital patients, in England and Wales, were significantly more likely to be white and female, annual incidence significantly rose to 0.53 cases per 100,000 in 2015. Laboratory confirmed patients in England and Wales were significantly more likely to be male, and annual incidence peaked in 2015 at 1.84 cases per 100,000. Hospital and laboratory cases could be described with high geographical resolution and displayed concordant areas of clustering in southern-central England.

Conclusions: This work will facilitate the development of alternative surveillance systems, economic assessments, and will impact the public health messaging and management of Lyme disease in the UK.
Title: Initial results from the Encephalitis cohort of the UK chimes study

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Abstract

Background: Outcomes in Childhood encephalitis can be improved by earlier patient identification and resultant expedited treatment.

Method: This analysis concerns clinical information on children identified as having suspected encephalitis from a larger population of patients recruited as part of the UK childhood meningitis and Encephalitis study (ChiMES). Initial analysis describes the patient body and looks to identify discriminators to assist in the early identification of patients with encephalitis.

Results: Of the 3009 children recruited as part of UK ChiMES 1589 met the suspected encephalitis criteria, of these: 277 patients were encephalitic, 1303 were non encephalitic & 9 non classifiable. The underlying aetiology of the 277 encephalitic children: 79 (28.5%) bacterial, 57 (20.6%), 68 (24.5%) autoimmune and 73 (26.4%) unknown. Associations found encephalitic patients were with higher age (median 3.4 years encephalitis vs. 0.3 years non encephalitis)
**Title:** Interactions of *Pseudomonas aeruginosa* with *Streptococcus* and *Rothia spp.* in an artificial sputum model

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**Abstract**

Objectives: A variety of microbes are present in the lungs of CF sufferers, including common pathogens, such as *Pseudomonas aeruginosa*. While interactions between *P. aeruginosa* and other well-studied CF pathogens such as *Staphylococcus aureus* are starting to be understood, little is known about how the species interacts with other commonly seen members of the CF lung community, such as *Streptococcus* and *Rothia spp.* We aimed to study these interactions in an artificial sputum model designed to mimic the conditions found in the CF lung.

Methods: *P. aeruginosa* LESB58 was inoculated into artificial sputum medium, in 24-well culture plates, either alone or in co-culture with *Streptococcus anginosus*, *Streptococcus vestibularis*, *Rothia mucilaginosa* or *Rothia dentocariosa*. All experiments were performed in triplicate. Samples were taken from 24hrs to 7 days after inoculation and assayed to assess growth. Gene expression was assessed using RNAseq.

Results: Differential expression of a variety of genes was seen in various co-culture conditions compared with single culture including genes related to prophage, type III secretion, exopolysaccharides and pyochelin production. No significant differences in *P. aeruginosa* growth rate were seen in co-culture with *Streptococcus spp.* compared to single species culture, but growth of *S. vestibularis* was significantly reduced after two days of culture.

Conclusions: The CF lung is a complex environment able to support a diverse array of microorganisms. A better understanding of interactions that alter the behaviour and virulence of pathogens in the CF lung may uncover new therapeutic targets and will help to inform future treatment strategies.