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Mucosal Perspectives in Pneumococcal Disease
Thursday 30 April 2009

Event venue: Liverpool Medical Institute

*A collaboration between the Infectious Disease Research Network
and the Liverpool School of Tropical Medicine*



<http://idrn.org/events/previous/pneumococci.php>

Report by Michael Head, Infectious Disease Research Network

Meeting summary by Adam Wright, Liverpool School of Tropical Medicine and
BRC National Institute for Health Research



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The organisers would like to thank the following companies for their support towards this event –



Note - Sanofi Pasteur MSD has provided an unrestricted educational grant to this workshop. They have not had any input into the arrangements or content of the workshop. The same also applies for GlaxoSmithKline Vaccines and Wyeth Vaccines.

About this event

This workshop was organised by the Infectious Disease Research Network (IDRN) with the scientific programme developed by the Liverpool School of Tropical Medicine and University College London. In particular, the IDRN would like to acknowledge the efforts of Adam Wright and Stephen Gordon (Liverpool), and Jeremy Brown (UCL).

We would also like to acknowledge the input of the speakers and workshop delegates in making the day such a success.

The aims of this workshop were to

- Identify priority areas for research focusing on the human mucosal respiratory immune response towards pneumococcal colonisation/infection
- Foster collaborations between research leaders for a focussed multi-disciplinary approach towards the development of novel interventions that will improve outcome for patients in the short and long term
- Lead to measurable outcomes in the form of funding proposals/submissions and published proceedings. The journal 'Vaccine' have indicated they would be keen to publish the proceedings of this meeting.

Post-workshop resource

The IDRN have created a webpage containing most of the presentations from the event, notes from the discussions, and also an email list of those who attended the workshop.

This resource is available at <http://idrn.org/events/previous/pneumococci.php>



Mucosal perspectives in pneumococcal vaccine development A one-day workshop

Venue: Liverpool Medical Institute

Date: Thursday 30th April 2009

Event webpage: <http://idrn.org/events/upcoming/pneumococci.php>

Organising Committee: Dr Jeremy Brown (UCL), Dr Stephen Gordon (LSTM), Michael Head (IDRN), Dr Adam Wright (Biomedical Research Centre, NIHR)

Programme

Arrival and Registration until 10.15

10.20 Welcome and Introduction

10.25 Session 1: Respiratory tract host defence vs pneumococcal carriage and disease (*Chair: Dr Neil French and Jeremy Brown*)

1. Acquired and innate host defence mechanisms in the respiratory tract – Known and Unknown (Dr Stephen Gordon, Liverpool School of Tropical Medicine)
2. Dynamics of pneumococcal carriage and host response(s) (Prof. Helena Käyhty, National Public Health Institute of Finland)
3. Mouse models of pneumococcal nasopharyngeal carriage and pneumonia - host pathogen interactions (Dr Aras Kadioglu, University of Leicester)
4. Host Mechanisms for Eradicating *Streptococcus pneumoniae* Pneumonia (Dr Jeremy Brown, UCL)
5. Dynamics between Virus and pneumococcal infection (Prof Tracy Hussell, Imperial College London)

12.45 Lunch

13.45 Session 2: Pneumococcal therapeutic strategies and correlates of protection
(*Chair: Dr Qibo Zhang and Dr Adam Wright*)

1. Pneumolysin - protein antigen and adjuvant (Prof Tim Mitchell, University of Glasgow)
2. Naturally acquired immunity in humans - modelling B & T cell responses to vaccine candidates in vitro (Prof Adam Finn, University of Bristol)
3. An in vitro model used to assess effects of nanoparticles on the epithelial airway barrier (Dr Martin Clift, University of Bern, Switzerland)

15.45 Tea and Coffee

16.15 Session 3: Translating Pneumococcal Research for Patient Benefit - Global and National perspectives (*Chair: Dr Stephen Gordon*)

1. Biomedical Research Centre Liverpool: Delivering Best Research for Best Health in Microbial Diseases (Prof Cheng Hok-Toh, Executive Director, Biomedical Research Centre, NIHR)
2. Protecting vulnerable groups with pneumococcal vaccine (Dr Neil French, London School of Tropical Medicine)
3. Identifying Research priorities Human / Protein / Adult / Child / Clinical Trials / Pneumococcus - what research in humans can tell us and what is possible with human studies

18.00 Closing Remarks and post-workshop drinks

N.B. Duration of talks: Session 1: 15-20 minutes plus 5 minutes questions
Session 2: 20 minutes plus 5 minutes questions
Session 3: 20 minutes plus 5 minutes questions

Delegates list

First name	Surname	Organisation
Aamir	Aslam	University of Oxford
Chris	Bailey	ImmBio
Paul	Balmer	Wyeth Vaccines
Mathieu	Bangert	University of Manchester
HELEN	BAXENDALE	University College London
Stephen	Bentley	Sanger Institute
Tobias	Bexten	
Andrew	Boyle	Sanofi-Pasteur
Jeremy	Brown	University College London
Isobel	Burnett	Calderdale and Huddersfield NHS Foundation Trust
Mary	Cavanagh	Imperial College London
Bambos	Charalambous	University College London
Sue	Clarke	ImmBio
Martin	Clift	University of Bern
Jonathan	Cohen	University College London
Rhett	Cooper	GlaxoSmithKline
Jacqui	Dawson	Wyeth Vaccines
Sherouk	El Batrawy	Liverpool School of Tropical Medicine
Claire	Entwisle	ImmBio
Vitor	Fernandes	University of Leicester
Daniela	Ferreira	University of Leicester
Jamie	Findlow	Health Protection Agency
Adam	Finn	University of Bristol
Stefan	Flasche	Health Protection Agency
Dona	Foster	University of Oxford
Linda	Franklin	University of Leicester
Neil	French	London School of Hygiene & Tropical Medicine
Duncan	Fullerton	Liverpool School of Tropical Medicine
Alexandra	Godlee	Imperial College London
Upali	Goonetilleke	Liverpool School of Tropical Medicine
Stephen	Gordon	Liverpool School of Tropical Medicine
John	Goulding	Imperial College London
Donna	Gray	Liverpool School of Tropical Medicine
Jenna	Gritzfeld	Liverpool School of Tropical Medicine
Lucinda	Hall	Queen Mary's, University of London
Mike	Head	Infectious Disease Research Network
Sue	Hill	ImmBio
Samia	Hussain	University of Leicester

Tracy	Hussell	Imperial College London
Cecilia	Jukka	Southport and Ormskirk Hospital NHS Trust
Aras	Kadioglu	University of Leicester
Helena	Käyhty	National Public Health Institute of Finland
Mohd. NadeemKhan		
Alun	Kirby	University of York
Maria	Koliou	Archbishop Makarios Hospital, Nicosia Cyprus
Marcus	Leung	University College London
Jiangtao	Ma	University of Glasgow
Carol	McInally	University of Glasgow
Paul	McNamara	Liverpool School of Tropical Medicine
Tim	Mitchell	University of Glasgow
Yaffa	Mizrachi Nebenzah	Ben Gurion University, Israel
Depesh	Pankhania	University of Leicester
Jeff	Pido	University of Bristol
Caroline	Pope	University of Bristol
Ana	Porras González	University Hospital Virgen del Rocío, Seville Spain
Elspeth	Potton	
Luke	Richards	University of Leicester
Kirsty	Ross	University of Glasgow
Jens	Rueggeberg	GlaxoSmithKline
Sarah	Smeaton	University of Leicester
Andrew	Smith	University of Glasgow
Elaine	Stanford	Health Protection Agency
Cheng-Hock	Toh	Liverpool School of Tropical Medicine
Mark	Wilks	Queen Mary's, University of London
Peter	Winstanley	Liverpool School of Tropical Medicine
Dan	Wootton	
Adam	Wright	Liverpool School of Tropical Medicine
Fatima	Wurie	Infectious Disease Research Network
Qibo	Zhang	Liverpool School of Tropical Medicine

Mucosal Perspectives on pneumococcal disease: A Meeting Summary
A one-day International workshop focusing on stimulating research and collaborations on this topic

Written by Dr Adam Wright, Biomedical Research Centre, NIHR, Royal Broadgreen and University Hospitals Liverpool

Date: Thurs 30 April 2009

Location: Liverpool Medical Institute

Website: <http://idrn.org/events/previous/pneumococci.php>

“Life is short, and Art long; the crisis fleeting; experience perilous and decision difficult” Aphorisms of Hippocrates represented on the Liverpool Medical Institute crest (*Trans. Francis Adams*)

Introduction:

The natural reservoir for *Streptococcus pneumoniae* (the ‘pneumococcus’) is the human nasopharynx and is believed to be a pre-requisite for mucosal (otitis media and pneumonia) and invasive disease (Bacteraemia and meningitis) [1]. Recent WHO estimates suggest that the pneumococcus is the leading cause for the majority of deaths in the under 5 age group worldwide, with the majority of these in sub-saharan Africa and Asia [2].

The dynamics between the nasopharyngeal mucosal immune system and microbial colonisers such as the pneumococcus need to be understood in order to develop reliable markers of vaccine efficacy and protection and also correctly formulate mucosal vaccines and/or adjuvants. Due to drawbacks with currently available polysaccharide based vaccines, efforts are underway within the research community to develop a new, possibly inhaled, vaccine(s) that will utilise pneumococcal proteins conserved across different polysaccharide serotypes, together with appropriate adjuvants for application into humans [3].

On a wet Thursday morning the doors of the Liverpool Medical Institute were opened to mark the opening of the workshop attended by 69 delegates from institutions across the UK, Switzerland, Finland, Cyprus, Israel and Malawi.

Proceedings commenced inside the auditorium with a welcome note delivered on behalf of the organising committee by Dr Adam Wright (BRC). Dr Wright emphasised the main aim of the day – ‘collaborative discussions’ hoping that the workshop would provide a stimulus towards the development of new and exciting research collaborations amongst the delegates. Michael Head (Infectious Disease Research Network) subsequently took the lead to highlight the role of the IDRN, specifically the promoting and fostering of new and existing collaborations, and the stimulation of multi-disciplinary research. In addition Mr Head thanked the sponsors – GlaxoSmithKline, Wyeth and Sanofi Pasteur - for their financial support in staging the workshop.

Session 1: Dr Stephen Gordon (Liverpool School of Tropical Medicine)

Dr Stephen Gordon opened the workshop. Pneumococcal epidemiology was summarised and particular attention was drawn to the difference between carriage and disease. Carriage precedes disease and is instrumental in transmission; there is an excess of both carriage and disease in children and adults with HIV infection. Basic mucosal defences against the pneumococcus were outlined and the key workshop themes with the speakers who would address these questions introduced:

- 1) The effect of pneumococcal carriage on the host and the effect of the host on carriage (i.e. epidemiology of the pneumococcus) (Helena Käyhty and Aras Kadioglu)
- 2) Understanding infection and host responses using murine models (Aras Kadioglu and Jeremy Brown)
- 3) Relationship between viral infection and subsequent pneumococcal disease (Tracy Hussell)
- 4) Vaccine candidates, adjuvants and measuring efficacy (Tim Mitchell, Adam Finn and Neil French)
- 5) Modelling lung immunity using an *in vitro* system (Martin Clift)

The remainder of the first talk addressed human studies of mucosal defence against the pneumococcus. Pulmonary defence is critically dependent on cellular and humoral defense. The key phagocyte in pulmonary surveillance is the alveolar macrophage [4, 5]. Alveolar Macrophages express receptors that can participate in opsonic and non-opsonic phagocytosis. Opsonic (Fc and complement receptor 3) and non-opsonic (MARCO) [6, 7] receptors have been demonstrated to be involved in the phagocytosis of the pneumococcus. Macrophage phagocytosis of opsonised pneumococci is intact in HIV infected adults. There is marked dysregulation of macrophage apoptosis in response to pneumococcal challenge and recent work has demonstrated the importance of macrophage turnover and efferocytosis in pulmonary defence [8]. Future attempts to prevent pneumococcal disease by manipulation of the pulmonary mucosal milieu will need to include consideration of the role of the alveolar macrophage in regulation as well as defense.

Professor. Helena Käyhty (National Institute of Health and Welfare, Helsinki, Finland)

Leading on from this Professor Helena Käyhty working at the National Institute of Health and Welfare presented an update on the PneumoCarr consortium consisting of Nine global partners. The aim of the PneumoCarr consortium is to develop the knowledge base and technical guidelines that would allow determination of vaccine efficacy to be based upon prevention of pneumococcal nasopharyngeal colonisation. It is envisaged that if this aim was to be achieved then prevention of nasopharyngeal colonisation could act as a surrogate or alternative benchmark for licensure of vaccines. Prof. Käyhty provided delegates with an authoritative and comprehensive account of the dynamics of nasopharyngeal carriage and what we understand about the immune response elicited by the host to control it. Prof. Käyhty suggested that there were 10^9 carriers globally, mostly in children and that one carrier harbours greater than 10^5 organisms at any one time. Most carriers are likely to harbour inter and intra-species variation and some may carry more than one serotype [9], from a total pool of 91 serotypes known to date. Helena presented evidence from two large epidemiological data sets (one in Denmark day care centres and the other from The Gambia) showing that pneumococci colonisers do not acquire new serotypes as readily as individuals who are/have not been colonised. In addition these studies demonstrated that the rate of clearance is similar regardless of the number of serotypes carried. An important caveat highlighted by Prof. Käyhty, however, is the sensitivity of the sampling methods employed and the sampling interval. Prof. Käyhty then proceeded to discuss the current data on the immune response to carriage. The current evidence suggests that the elicitation of antibodies, offers protection against systemic disease but little protection at the mucosal surface. Important work carried out in the US (led by Marc Lipsitch and Richard Malley) [10, 11] and in the UK by Aras Kadioglu [12] has demonstrated that protective immunity to pneumococcal carriage in murine models is mediated by CD4 antigen specific T cells, particularly those producing IL-17. Our understanding of the T cell response to

the pneumococcus has grown in recent years in the wake of a better understanding of the roles and functions of T cell subsets particularly Interleukin-17 producing T helper (Th₁₇) cells. In humans recent work suggests that there may be similar mechanisms of protection against the pneumococcus [10, 13]. More experimental human studies need to be undertaken however to verify these interesting findings and allow for better vaccine design and choice of adjuvant.

Dr Aras Kadioglu (University of Leicester)

Animal models are important to inform experimental human studies and for the design of vaccines. Dr Kadioglu has set up mouse models to address questions relating to pneumococcal colonisation and acute or chronic mucosal infection. These models are used to provide information on the contribution made by individual pneumococcal virulence proteins towards establishing colonisation/infection and also on the host immune response at a given *in vivo* locality. Dr Kadioglu pointed out that the contribution of individual pneumococcal virulence factors (and thus the immune response) towards colonisation/infection differs individually and collectively and is dependent on the *in vivo* location of the bacterium and host genetics [14, 15]. Dr Kadioglu presented new data showing the establishment of long-term asymptomatic pneumococcal nasopharyngeal carriage in a mouse model that was followed for a period of 28 days. To maintain carriage, pneumococcal pneumolysin is important since without it the host is able to clear nasopharyngeal carriage within 7 days. Dr Kadioglu subsequently highlighted other pneumococcal proteins that also contribute to colonisation/infection [16] before focusing on the role of PavA (pneumococcal adherence and virulence factor A). Dr Kadioglu presented interesting new data demonstrating the importance of Pav A for long-term asymptomatic nasopharyngeal carriage and its role in translocation across host tissue barriers during bacteraemia. The mechanism of PavA virulence is currently under further investigation.

Dr Jeremy Brown (University College London)

The discussion subsequently turned its focus away from the pneumococcus and towards the host immune response. Dr Brown provided an overview of the immune mechanisms that may provide protective immunity early and late in the course of lung infection and highlighted the multi-factorial nature of the host response to the pneumococcus. In contrast to Dr Gordon's discussion on the role of immunoglobulin, Dr Brown also emphasised the role of complement as a mediator of the innate and adaptive immune response through opsonising the pneumococcus for uptake by macrophages. Given the relatively low concentrations of complement present in alveolar lining fluid it remains to be established what role complement plays in human lung mucosal immunity against pneumococci. The effect of complement is likely to vary between individuals and different pneumococcal isolates and these may be factors influencing susceptibility to pneumococcal infection.

Following phagocytosis macrophages are able to act as 'gate-keepers' of the immune response by initiating pro-inflammatory responses such as tumour necrosis factor in response to a large bacterial challenge, or having an anti-inflammatory effect in response to a smaller bacterial challenge through apoptosis [4, 5, 8]. Dr Brown highlighted studies demonstrating the importance of defective signalling pathways of the Toll-like receptors (TLR) for protection of both mice and humans against pneumococcal infections [17]. The lung inflammatory response to the pneumococcus results in phagocyte recruitment, with a marked influx of neutrophils and monocytes into the lungs. Neutrophils are able to phagocytose pneumococci and this is also largely complement-dependent, but the speaker reminded delegates that the role of the neutrophil is uncertain since patients with either chronic granulomatous disease or neutropenia have only a moderate increase risk of pneumococcal infection. Other protective mechanisms that have come to light recently include the role of the Th17 subset, which have been shown to be important

for acquired immunity to nasopharyngeal colonisation with the pneumococcus and may have a similar role against lung infection [11]. T cell function (CD4 and CD8) could be important for future vaccine strategies since they can provide immunological memory if stimulated appropriately, and these memory responses can be measured using whole blood or cells isolated from the respiratory tract. Dr Brown concluded that different immunological protective mechanisms operate at different stages of pneumococcal colonisation and infection. It is currently unclear whether an intervention to eradicate colonisation or prevent mucosal infection in the lungs would be the best strategy. Aiming to alter the microbial flora of the nasopharynx to eradicate the pneumococcus may not be beneficial, as the recent introduction of the conjugated vaccine has demonstrated that strains not eradicated by the vaccine can quickly refill the ecological niche.

Professor Tracy Hussell (National Heart and Lung Institute, Imperial College London)

On the topic of lung immunity we were reminded by Professor Tracy Hussell of the site specific immunoregulatory mechanisms that are in place to prevent over exuberant pro-inflammatory immune responses within the lung. Overcoming such mechanisms by short term stimulation is a challenge to the design and application of protein based anti-pneumococcal vaccines within the lung. Further challenges include the impact concomitant viral infection and the role an anti-viral response may play towards predisposing towards pneumococcal infection. Prof. Hussell discussed the 'immune rheostat' concept whereby 'brightening' and 'dampening' pathways operate concomitantly to regulate innate immune activation within the lung. In this area Prof. Hussell demonstrated the important role of CD200 expressed by T cells and epithelial cells and its interaction with its receptor found at high levels on alveolar macrophages and granulocytes [18]. Ligation of CD200R with its ligand can attenuate pro-inflammatory cytokine production by macrophages. Recently it has become increasingly apparent that not only can innate immunity halt pro-inflammatory mechanisms mediated by adaptive immune cells but the converse is also true [19]. Elucidating the regulatory mechanisms of this bi- or even multi-directional feedback loop will provide an important framework for the testing of vaccine adjuvants. As an important caveat to this Prof. Hussell highlighted the dangers of sub-optimally adjusting mucosal immunity.

Prof. Hussell provided a prescient reminder of dysregulated mucosal defences using epidemiology data from the 1918-1919 influenza pandemic. This demonstrated that subsequent bacterial pneumonia due to the pneumococcus was a major cause of mortality [20]. The recent identification of swine flu infected persons in Mexico and the speed with which global transmission of disease can occur was a timely demonstration on the importance of this topic. Using murine models of infection Prof. Hussell demonstrated that viral infection for three days prior to bacterial infection resulted in an inability of the host to reduce the bacterial burden in the airways culminating in a much earlier death. The immune response following viral challenge was still compromised 4-6 weeks later since pneumococcal challenged mice had impaired clearance of airway and lung bacteria. The immune profile following a previous infectious episode can have an important bearing on subsequent immune responses to infection or vaccine application as Prof. Hussell and other groups have demonstrated [21]. Biomarkers may thus have to be developed to ensure that a deleterious or ineffectual immune response will not ensue following vaccine administration. An example of this could be the levels of CD200R on macrophages. Here Prof. Hussell noted that 14 days post-viral infection there was a higher basal level of CD200R expression on alveolar macrophages suggesting a heightened threshold for triggering of an immune response. Clearly further research is required in this area.

Session 2: Professor Tim Mitchell (University of Glasgow)

The second session, chaired by Dr Adam Wright and Prof. Qibo Zhang, moved away from the host-pathogen interaction and towards vaccine candidates and measuring immune responses following vaccine administration. Professor Tim Mitchell provided an interesting talk regarding the use of the pneumococcal virulence factor pneumolysin as a candidate protein vaccine and adjuvant. The advantages of using pneumolysin are that it is a conserved protein, expressed by all invasive serotypes and it plays an important role during pneumococcal colonisation/infection [16]. Prof. Mitchell emphasised the important caveats to this, which include its cytotoxicity to mammalian cells and that on its own it can reproduce pneumococcal disease symptoms in murine lungs. For this reason (and to understand pneumolysin biology) non-toxic forms ($\Delta 6$) have been developed to abrogate its toxicity towards mammalian cells. A vaccine composed of a fusion of $\Delta 6$ and capsular serotype 4 gave full protection (measured as survival and bacterial load) to mice challenged intra-peritoneally with a serotype 4 pneumococcal strain compared to either alone. The fusion of $\Delta 6$ or its wild type counterpart –pneumolysin – to known pneumococcal virulence proteins for use as a vaccine is an attractive avenue of research that is currently being pursued in his laboratory. Pneumolysin can thus act as an adjuvant and antigenic target. In line with this, Prof. Mitchell was able to demonstrate good mucosal IgA responses following vaccination of mice with fusion proteins compared to single administration.

Five pneumococcal antigens (PsaA, PspA, PspC, PhtD and Mixture) are part of a PATH project to find the best fusion mixture that would give the best protection in murine models of pneumococcal colonization and pneumonia. Vaccines based on single conserved pneumococcal proteins have elicited protective responses from mice however this could ultimately be improved by conjugating several together. In addition, for a mucosal application, an adjuvant will be necessary given the immunoregulatory mechanisms that exist, as pointed out by Prof. Hussell, at the mucosal surface. A non-toxic version of pneumolysin would be ideal. Current limitations appear to be confined to highlighting appropriate antigens and chemically coupling them together. Prof. Mitchell stressed that this work is in a very early stage. One fusion protein that is undergoing evaluation is PsaA-PLY. Intranasal administration of PsaA- $\Delta 6$ or PsaA-PLY into mice elicited 10-100-fold more PsaA specific IgG, respectively, compared to PsaA alone. These early studies suggest that the PsaA- $\Delta 6$ construct is more effective than PsaA alone but 50% less than that carrying the wild type pneumolysin protein. Reducing the gap in effectiveness between fusion constructs would be an important step towards producing an effective construct for human trials. Important questions that generated debate were ‘Which protein(s)?’ and ‘What type of response(s)?’ One pertinent question was what is the next step to take these protein constructs closer into humans?

Professor Adam Finn (University of Bristol)

The focus subsequently shifted from the composition of new vaccines to modulation of the immune response by adjuvant, e.g. bacterial lipoproteins, delivered by Prof. Adam Finn from Bristol. Bacterial lipoproteins are Toll-like receptor 2 agonists and their effect on pneumococcal specific memory and naive adenoidal B and T cell responses were discussed. They demonstrated that adenoidal mononuclear cells from pneumococcal culture positive children produce high levels of anti-pneumococcal protein antibodies when stimulated (“memory response”) with pneumococcal proteins. In contrast antigen specific immunoglobulin towards the pneumococcus could not be detected in the culture supernatant of the adenoidal mononuclear cells from approximately half of the culture negative children (“Naive

response”). The next question was whether trace amounts of antigen specific immunoglobulin could be increased if adenoidal mononuclear cells were stimulated in the presence of a TLR 2 agonist. Interestingly, whilst the naive responses increased dose dependently with TLR 2 agonist concentration, memory responses declined by over half setting up an interesting dichotomy. This outcome was also reflected in memory T cell proliferation data. Here proliferation of antigen specific CD45RO T cells declines in the presence of a TLR 2 agonist whereas the proliferation of CD45RO depleted cells (i.e. naive cells) increases. Professor Finn provided evidence that increased IL-10 production and ICOS expression may be down-regulating this memory response via production from antigen presenting cells and expression on T cells, respectively. Drawing on data presented earlier in the day particularly that demonstrated by Prof. Hussell, TLR 2 agonists may be a useful tool to return the immune rheostat following viral infection or vaccination towards its normal anti-inflammatory homeostatic state to prevent unwanted inflammation. It was made clear that more information about immune regulatory mechanisms in health and disease is required prior to attempting to modulate responses via adjuvants but nevertheless it has provided us with some useful insights.

Dr Martin Clift (Institute of Anatomy, University of Bern)

Dr Martin Clift wrapped up the post-lunch session with an entertaining and informative presentation on the effect of nanoparticles (NPs) on the epithelial airway barrier. Martin offered two definitions of what constituted a nanoparticle: 1) particles with at least one dimension <100nm [22] and 2) a nano-object (a material with one, two or three external dimensions in the nanoscale) with all three dimensions in the nanoscale¹ and further described how the Institute of Anatomy, at the University of Bern, have constructed an *in vitro* model of the epithelial airway barrier. The model, presented by Dr Clift, is the culmination of work in the laboratory of Dr Barbara Rothen-Rutishauser and Professor Peter Gehr. The triple cell co-culture model reflects three different cell types of the epithelial airway barrier; 1) airway epithelial or bronchial cells forming the membrane barrier, 2) monocyte derived dendritic cells attached to the basal side and 3) monocyte derived macrophages which were located on the luminal side [23]. Although alternative *in vitro* co-culture systems of the lung exist [24], the model by Dr Rothen-Rutishauser reflects exactly the *in vivo* juxtaposition of cells as well as takes into consideration the interaction and cumulative cellular responses rather than examining the response of single cell types. In addition to the *in vitro* co-culture, collaboration with the Helmholtz Centre in Munich, Germany, has enabled the Institute of Anatomy to further research the effect of NPs at the air-liquid interface via forming aerosols which are delivered onto the triple co-culture system. These developments are extremely interesting since if translational science is to be successful then not only are good animal models required but also an improvement and refinement in laboratory human models that closely reflect interactions *in vivo* are also required. The pipeline of promising animal vaccine research to early phase human trials can then increase with the aim of improving patient care. The triple cell co-culture model is currently being used to evaluate the potential harmful effects of NPs on the epithelial airway barrier. Although much is known as to the harmful effects of nanoparticulates contained within air-pollution (formed from processes such as exhaust fumes) it is not yet clear what the full effects are following exposure to these accidental NPs. Also, at this moment in time, it is unclear whether manufactured NPs, such as those intended for use as medical applications, have deleterious effects on human health and the environment. Dr Clift and his colleagues at the Institute of Anatomy in Bern, as well as their world-wide collaborators, are currently researching these many aspects in

¹ International Standards Organisation (ISO) Technical Specification (ISO/TS) 27687; Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate; First published 2008-08-15.

order to provide information as to the potential harmful effects that could occur following exposure to NPs, and subsequently what the consequences of these exposures could be following NP/cell interaction.

Session 3:

Professor Cheng-Hok Toh (Biomedical Research Centre, NIHR)

Professor Cheng-Hok Toh briefly took the stage to describe the exciting developments occurring in Liverpool as one of the few Biomedical Research Centres (BRCs) in the UK. BRCs arose from the conclusion of the Cooksey review (December 2006) for the House of Commons Science and Technology select committee. The review highlighted insufficient translation of basic into applied research culminating in insufficient applied research to meet health needs. The NHS along with University and industry forms a central part to the increased output of translational research. This has been exemplified by many of the discussions held today, which have shown the current extent of inter-institutional co-operation. Professor Toh covered some of the strengths of the Liverpool bid and the themes, which Liverpool are leading on. The four themes based at Liverpool include 1) Safety of anti-microbials 2) Sexual Health 3) Community and Hospital Inquired Infections and 4) Pulmonary Infections. These themes are measured against milestones to assess progress by an external advisory panel. Finally Prof. Toh highlighted the clinical research facility and repository that has recently been set up within the Liverpool Hospitals NHS trust, University of Liverpool and Liverpool School of Tropical Medicine. His take home message was that Liverpool and the BRC are ready to take on the challenges to meet the local and global health needs of the 21st century.

Dr Neil French (London School of Hygiene and Tropical Medicine)

The aim of the final session was to get an insight into pneumococcal vaccination “in the field” kindly delivered by Dr Neil French who had travelled to the workshop from Malawi. Subsequently there was an open floor discussion about the next steps that need to be taken to reach the goal of protecting vulnerable groups of pneumococcal mucosal disease here in the UK and overseas. Dr French highlighted the push by advocacy groups to introduce seven, ten and thirteen valent pneumococcal conjugate vaccines (PCV-7, 10, 13) to vulnerable groups mostly in sub-saharan Africa and Asia. Dr French subsequently provided evidence that PCV-7 has showed efficacy in protecting against invasive and mucosal disease in target groups (e.g. HIV infected Malawians).

Returning to the theme covered by Prof. Mitchell earlier in the day, new vaccines are required to surmount limited serotype coverage, limited protection at mucosal sites and durability posed by the current conjugate vaccines. Dr French discussed the challenges that are presented when introducing a new vaccine particularly those not based on pneumococcal capsule and when no *in vitro* correlate of immunity can be used as a surrogate. A randomised control trial will be necessary however it is unclear what is an appropriate control and end-point? Is the sample size attainable particularly when comparing with the current gold standard of a multivalent protein conjugate capsular vaccine? What end-points should be measured clinically and immunologically? How will the immunological endpoints be confounded by carriage? Hopefully PneumoCarr will be able to shed light on these latter topics and we look forward to hearing about their work at future meetings such as the International Symposium on pneumococci and pneumococcal disease to be held in Israel in 2010 (ISPPD-7).

Discussion Session – Chair Dr Stephen Gordon

Dr Gordon asked all of the speakers to return to the front of the audience for their thoughts on some of the challenges facing mucosal pneumococcal research and to answer any questions from the audience. The panel included John Goulding (representing Prof. Hussell), Dr Martin Clift, Dr Neil French, Prof. Helena Käyhty, Prof. Tim Mitchell and Dr Aras Kadioglu. The aim of this session was to identify areas of the host immune response and pneumococcal biology that require further study. It was noted that there are some important questions regarding the host-bacterium relationship that require consideration in the development of a mucosal vaccine including:

- a) What is the population size for the pneumococcus at the nasopharynx during health and during invasive disease?
- b) What are the consequences of 'good' mucosal immunity?
- c) What are the contributors for the transition from a 'harmless' coloniser to invasive disease?

A lively discussion ensued on this latter topic highlighting the multi-factorial and dynamic nature of the host- opportunistic pathogen relationship. Possible contributing factors included the initiation of biofilm formation (how important is this process to disease formation in humans? Should more attention be directed towards it?), contribution made by co-coloniser strains/serotypes not usually assessed? Which contributes more the genetics of the host or the pneumococcus?

Questions posed by the workshop included:

- What human experimental medicine studies are required that will translate basic pneumococcal research for patient benefit?
- Should the aim of new vaccines be the prevention of carriage?
- What are the consequences of reduced pneumococcal carriage?
- Which pneumococcal proteins should be taken forward for a mucosal vaccine?
- Which adjuvant and what is the most appropriate response?
- How do we evaluate new (probably protein) vaccines against the current 'gold-standard'?

To sum up this final session the chair person asked the speakers what would be their ideal project.

From this the following themes emerged:

- A greater multi-disciplined approach between microbiologists and immunologists whilst tracking a single pneumococcus and its interaction within the nasopharynx
- A full Genomic approach directed towards the pneumococcus during the transition between colonisation and infection.
- What are the effects of nano-particle exposure on predisposing to subsequent colonisation and infection.
- How protein vaccines work with the aim of developing functional lab immunological assays to determine efficacy following administration

Clearly there are many unanswered questions on this topic and it is hoped that over the coming years light shall be shed on these important questions.

Acknowledgments:

Our acknowledgments also go to Fatima Wurie (IDRN), Sam Taylor-Fletcher (LSTM) and staff of the Liverpool Medical Institute- their assistance was much appreciated. Also our thanks go to the sponsors Wyeth, GlaxoSmithKline and Sanofi Pasteur for their financial support.

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