MEETING REPORT

Mucosal Immunology Down Under: Special Interest Group in Mucosal Immunology Workshop, Australasian Society for Immunology, Sydney, Australia, 2 December 2007

Allan W Cripps^{1*}, Philip Sutton², Ken Beagley³, Sarah Robertson⁴, Margaret Dunkley⁵

¹Griffith Health, Griffith University, Gold Coast campus, QLD, Australia, 4222

²Centre for Animal Biotechnology, School of Veterinary Science, University of Melbourne, VIC, Australia, 3010

³Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove Campus, Brisbane QLD, Australia, 4059

⁴Research Centre for Reproductive Health, Discipline of Obstetrics & Gynaecology, University of Adelaide, SA, Australia, 5005

⁵Hunter Immunology Ltd, University of Newcastle, Callaghan NSW, Australia, 2308

Keywords: chlamydia, common mucosal immune system, gastrointestinal immunity, *Haemophilus influenzae*, *Helicobacter pylori*, M cell targeting, middle ear, otitis media, reproductive immunity, respiratory immunology, vaccine

*Corresponding Author

Allan W Cripps

Griffith Health, Griffith University

Gold Coast campus

QLD Australia 4222

Phone: 61 7 5678 0709

Fax: 61 7 5678 0795

Email: allan.cripps@griffith.edu.au

The Mucosal Immunology Special Interest Group (SIG-MI) of the Australasian Society of Immunology (ASI) was formed 14 years ago and has run regular symposia and workshops in conjunction with the ASI since that time. In December 2007 the SIG-MI held a one day satellite workshop in conjunction with the annual ASI scientific meeting in Sydney to celebrate the decade since hosting the 9th International Congress of Mucosal Immunology (9-ICMI) in 1997 which was also held in Sydney. The meeting which was attended by 65 delegates focussed on 4 session themes: reproductive immunology, respiratory immunology, mucosal immunology of the gastrointestinal tract and mucosal vaccines.

REPRODUCTIVE IMMUNOLOGY

The plenary speaker, Professor Charles Wira (Dartmouth Hitchcock Medical Center, USA), provided an overview of how the female sex hormones estradiol and progesterone regulate both innate and adaptive immunity in the female reproductive tract to meet the unique requirements of accommodating an immunologically distinct fetus and protecting against sexually transmitted viral and bacterial pathogens. He provided evidence that innate immunity may provide the major means of protection against infection at the time of the reproductive cycle when adaptive immunity is down regulated by sex hormones to facilitate successful reproduction. The need to regulate the adaptive immune system in the female reproductive tract for successful reproduction may provide a window of vulnerability to sexually transmitted pathogens such as HIV and must also be considered when evaluating the effectiveness of vaccines against sexually transmitted pathogens where vaccine-induced responses may also be affected by sex hormone regulation.

The use of bioinformatics approaches for in silico identification of novel protective chlamydial antigens was discussed by Professor Ken Beagley (Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia). Using two web-based T cell epitope prediction programs, chlamydial T cell epitopes that were conserved across the two major human chlamydial pathogens *Chlamydia trachomatis* and *C. pneumoniae* and that bound several MHC-II types, were identified. Immunisation of mice with one of these antigens (ribonucleotide reductase small chain protein, NrdB) elicited chlamydia-neutralising antibodies in serum and genital tract secretions. Adoptive transfer of NrdB-specific CD4 T cells to naïve mice provided partial protection against genital chlamydia infection (Barker et al. in press). Combinations of novel antigens are currently being evaluated in animal models of chlamydial infection.

Continuing the chlamydia theme Alison Carey (Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia) reported on how the timing of chlamydial vaccination might affect the clearance of Chlamydia following a genital challenge infection. Using previously established vaccination regimes (Berry et al, 2004; Skelding et al 2006), naïve mice, mice that had recovered from a chlamydial infection or mice that were at the peak of an ongoing genital infection were immunised with the chlamydial major outer membrane protein (MOMP) mixed with the adjuvants cholera toxin and CpG. Immunisation during an ongoing chlamydial infection did not affect recoverable chlamydial counts or rates of clearance compared to infected, non-immunised mice, however the degree of hydrosalpinx was reduced in intranasally immunised animals. The results suggest that chlamydial vaccination may be most effective in chlamydia-naïve individuals and that the infection status of an individual may influence the degree of protection.

Amanda Yeung (Department of Pathology, University of Sydney, Australia) demonstrated the importance of interferon gamma (IFN γ)-induced indoleamine 2,3-dioxygenase (IDO) production in the clearance of viral STDs. Vaginal infection of mice with West Nile Virus (WNV) was of greater magnitude in both IFN γ ^{-/-} mice and mice with pharmacological inhibition of IDO production, compared to controls. In normal mice, clearance of WNV coincided with the peak of IDO production. Also demonstrated was the fact that pharmacological abrogation of IDO enhanced infection with an attenuated Herpes simplex virus type 2 (HSV-2) strain. Indeed, infection of mice with wild type HSV-2 at the time of peak WNV-induced IDO production significantly reduced HSV replication and prolonged survival compared to control mice. The data demonstrate the important role of IDO in anti-viral immunity.

Associate Professor Mark Hedger (Monash Institute of Medical Research, Monash University, Australia) reported on inflammatory cytokine production by rat Sertoli cells and testicular macrophages in response to stimulation with TLR4 and TLR2 ligands, LPS and Pam3Cys respectively. Sertoli cells produced significantly less IL-1 α or IL-6 than macrophages but produced 20-fold more activin A when stimulated with LPS. While expression of TLR4 and MD2 was comparable between Sertoli cells and macrophages, CD14 and TLR2 expression on Sertoli cells was reduced compared to macrophages. Ligation of TLR2 on Sertoli cells by Pam3Cys elicited the production of both IL-1 α , IL-6 and activin A, which was sustained for up to 12 hours. Production of activin A by Sertoli cells was blocked by inhibitors of p38 and Jnk MAP kinases but not by NF κ B inhibitors. Signalling through TLRs on Sertoli cells may be an important means of communication within the seminiferous epithelium under normal conditions and may contribute to testicular failure during inflammation (Girling and Hedger, 2007).

Associate Professor Sarah Robertson (University of Adelaide, Australia) reported on studies to identify the nature and role of seminal fluid signalling molecules that influence the immune response in the female reproductive tract. This is essential to understand how the female tissues discriminate and respond appropriately to spermatozoa compared with infectious micro-organisms (Robertson 2005). All three mammalian isoforms of TGF β are present in high concentrations in seminal plasma, and their interaction with cervical epithelial cells after insemination causes a local inflammatory response in the cervical tissue. Using Affymetrix microarray and qRT-PCR in an *in vitro* model system (Sharkey and Robertson, 2007), TGF β was shown to account for most but not all of the pro-inflammatory signalling activity in seminal fluid. The cervical response is characterised by cytokine and chemokine expression and recruitment of inflammatory leukocytes, and thus seminal fluid TGF β content might influence susceptibility to infection by leukocyte trophic viruses such as HIV, as well as competence of the innate and adaptive immune compartments to tolerate the male gametes as well as protect from sexually transmitted diseases.

RESPIRATORY IMMUNOLOGY

An understanding of the anatomy of the lung, and the contribution made by responses generated in different lymphoid tissues is fundamental to an understanding of respiratory immunology and how mucosal immune responses contribute to protection in the respiratory tract. Professor Reinhard Pabst (Department of Functional & Applied Anatomy, Medizinische Hochschule Hannover, Germany) has been an important contributor in this area and is one of a group who proposed use of the term ‘the integrated immune system’ for what was previously termed the

‘common mucosal system’ in order to more accurately describe the interaction between different mucosal sites (Brandtzaeg et al, 2008).

At the Mucosal Workshop, Professor Pabst described the distribution of lymphocytes in different organs contributing to a total of approximately 490×10^9 cells in the human adult. The lung contains approximately 30×10^9 , gut (including Peyer’s Patches) approximately 50×10^9 , and blood 10×10^9 with approximately 1/3 of these in the pulmonary marginal intravascular pool (Blum and Pabst 2007). Bronchoalveolar lavage is now commonly sampled in humans and cells that enter the airways are now known to migrate to local lymph nodes and are not just removed by the muco-ciliary escalator. Effects of macrophage activating lipopeptide (MALP-2) were also presented. Intra-tracheal delivery of MALP-2, a ligand for TLR2 and TLR6, leads to an increase in leukocyte infiltrate and can reduce lung metastases. MALP-2 application after ovalbumin (OVA) challenge in an OVA allergy model leads to an increase in neutrophils, T-reg and dendritic cells (DC) in the lung. Different effects of MALP-2 were observed in young (weaned) and adult rats (Luhmann et al 2007). An important leukocyte compartment also occurs in the peri-arterial space in different diseases but the reason for this is not known. In studies of human lungs following fatal asthma attacks the perivascular space has many eosinophils and mast cells. There are species differences with rats and humans, but not mice, having an arterial blood supply to the bronchial wall. The need for examination of all lung compartments containing lymphocytes when assessing allergic or pathogenic responses was emphasised.

Associate Professor Margaret Dunkley (Hunter Immunology Ltd, University of Newcastle, Australia) presented results of Phase 1 and 2 clinical studies with a killed whole-cell non-typeable *Haemophilus influenzae* (NTHi) oral vaccine (HI-

164OV). In the 3-month Phase 1 study, smokers (10 cigarettes per day for the past two years), were administered HI-164OV or placebo tablets on days 1-3, 29-31 and 57-59. Thirty placebo and 30 active treatment subjects completed the study. Examination of NTHi-specific lymphocytes in blood by *in vitro* stimulation with NTHi antigen, showed an increase throughout the study in the placebo group suggesting induction of NTHi-specific T cells as a result of exposure to live NTHi. The HI-164OV vaccine boosted this naturally-occurring response. The serum NTHi-specific IgA response fell in the placebo group and rose slightly in the active group. The serum and saliva NTHi-specific IgG remained unchanged in the active group but was higher and more variable in the placebo group suggesting this was due to exposure to live NTHi that reached the lower airways and that this was being prevented in the active treatment group. The relationship between exposure to live NTHi and serum NTHi-specific IgG in the placebo group was further demonstrated by the fact that placebo subjects with higher exposure to NTHi (as measured by number of visits at which NTHi was detected in gargle samples) had a significant increase in NTHi-specific IgG and those without such exposure to live NTHi did not. Efficacy of HI-164OV was indicated by a significant reduction in use of concomitant medication in the active treatment group.

In the Phase 2 studies, subjects were recruited on the basis of having 2 or more acute bronchitis episodes per year for the past 2 years and on the basis of FEV1 were allocated to Study HIH004 (Mild to moderate, FEV1 > 50% of the predicted value) or Study HIH002 (Moderate to severe, FEV1 ≤ 50% of the predicted value). In each study, subjects were randomly allocated to receive medication or placebo. The number of subjects randomised and analysed was 38 for HI-H002 and 102 for HI-H004. These studies demonstrated that HI-164 reduces the severity of acute

exacerbations, assessed by hospitalisation, and usage of medication (antibiotics, steroids, bronchodilators). Subjects with moderate to severe COPD had particular benefit compared to those with mild airway disease and a major reduction in hospitalisation (of 90%) due to respiratory events seen in the moderate-severe disease group, identifies oral immunisation with HI-164OV as a distinctive novel therapy for patients with COPD.

Mechanisms of airway hyper-responsiveness (AHR) were also addressed in this theme. Dr Matthew Wikstrom (Faculty of Medicine, Dentistry and Health Sciences, The University of Western Australia, Australia) described OVA capture and processing in main airway tissue of allergic mice using OVA labelled with red or green fluorescent dyes and flow cytometry. Macrophages and CD11b^{hi} DCs captured labelled OVA in naïve mice, but in mice immunized by two intra-peritoneal injections of OVA in aluminium hydroxide, macrophages, DCs and B lymphocytes all exhibited a marked increase in their capacity to capture and process inhaled OVA (von Garnier 2007). To determine what mechanisms drive AHR, OVA aerosol was administered and samples taken at 0, 2, 12, 24 and 48h. CD40 was transiently upregulated on CD11b^{lo} dendritic cells at 12h, coinciding with the earliest increase in CD4⁺ T cell activation and preceding the induction of AHR. This posed the question: does interaction between DCs and T cells drive AHR? This question is still being examined but the conclusions from these studies were that allergic sensitization increased antigen uptake and processing, and was mediated by specific antibody because the activity was antigen-specific and could be transferred to naïve mice by serum transfer. IgG1 appears to be responsible for enhanced antigen uptake in this model since a role for IgE was ruled out when heat-inactivation had no effect on opsonisation.

When microbes encounter the host immune system a number of decoy mechanisms are activated. Dr Bruce Wines (Burnett Institute, Victoria, Australia) discussed how the success of *Staphylococcus aureus* as a human pathogen is dependent on evasion of the immune response. Loops 1 and 4 of the OB (oligosaccharide/oligonucleotide-binding) domain of the superantigen-like protein, SSL7, bind to the IgA Fc. On the IgA Fc, the CH2 domain α 1-helix and the CH3 domain FG loop together to form the major binding interface (Ramsland et al 2007). The myeloid receptor Fc α R1 also binds to the IgA Fc CH2/CH3 domain interface and so SSL7 competes with Fc α R1 for IgA binding. Episodes of natural selection have shaped the interaction of IgA-Fc with Fc α R1 and bacterial decoy proteins, (SSL7, *S. aureus*; M protein, group A streptococci; β -protein, group B streptococci) in an arms race between bacterial pathogens and the host immune system. Serum IgA can enhance phagocytosis, respiratory burst and cytokine secretion. Secretory IgA is non-inflammatory but can induce respiratory burst. IgG is possibly more important for systemic *S. aureus* infection but what about mucosal infections? One third of the population has nasal colonisation with *S. aureus* and this predisposes to systemic *S. aureus* infection. In conclusion, 3 questions were posed: Does SSL7 binding of IgA enhance *S. aureus* infection, does the nasal IgA response correlate with infection or protection, and what protective or non-protective polymorphonuclear leukocyte functions are triggered by *S. aureus*-specific IgA?

Microbial interactions in otitis media (OM) were presented by Ajay Krishnamurthy (Central Queensland University, Australia) with a study of the microbiology and inflammatory responses in a mouse polymicrobial OM model. Prominent microbes in OM are *Streptococcus pneumoniae*, *Moraxella catarrhalis* and NTHi. A polymicrobial model of OM was developed to investigate how concurrent

infection with these bacteria with, or without Sendai virus, may affect OM incidence and severity. BALB/c mice were infected intra-nasally with one, two or three bacterial species with and without virus administered 3 days previously. Nasal and middle-ear lavages were performed to determine bacteria levels, and leukocyte numbers and NO were assessed in middle ear lavage fluid. The study demonstrated that co-existence of different bacteria caused early onset of OM, the inflammatory cascade was not triggered until Day 3 in single bacteria infection, but early onset was seen in the polybacterial environment. The presence of *M. catarrhalis* affected *S. pneumoniae* recovery in both nasopharynx and middle ear. Prior infection with Sendai virus enhanced bacterial recovery from the middle ear but not the nasopharynx. An early inflammatory response was observed (polymicrobial infection) in the absence of recoverable bacteria in middle ear, suggesting that normal bacterial culture may not be sufficiently sensitive, as seen in acute OM and chronic serous OM where there is persistent inflammation with limited bacterial recovery.

MUCOSAL IMMUNOLOGY OF THE GASTROINTESTINAL TRACT

Innate defences

Recent years have seen considerable advances in our understanding of the induction and regulation of inflammatory responses to pathogenic infection of the gastrointestinal mucosa. In particular, the major contribution of the innate immune system in these processes was only relatively recently fully appreciated. For example, Nod1, a cytoplasmic molecule which recognises peptidoglycan from pathogenic Gram negative bacteria, is now recognised as an important mediator of inflammation. While TLR are key recognition molecules for many pathogens, they appear of limited importance for inducing inflammation in response to infection with the human gastric

mucosal pathogen, *Helicobacter pylori*. This is largely due to *H. pylori* possessing modified pathogen-associated molecular patterns such as lipopolysaccharide and flagellin which are poor activators of TLR4 and TLR5 respectively. Instead, the laboratory of Dr Richard Ferrero (Department of Microbiology, Monash University, Australia) has identified that Nod1, present within epithelial cells, acts as an important recognition system for *H. pylori* peptidoglycan. They have demonstrated that Nod-1 activation can result from peptidoglycan delivered into gastric epithelial cells by a type IV secretion system and that this activation triggers an inflammatory response via a pathway involving NF- κ B (Kaparakis et al., 2007; Viala et al., 2004). Dr Ferrero provided new evidence that bacterial outer membrane vesicles, not only from *H. pylori* but also from *Neisseria gonorrhoea* and *Pseudomonas aeruginosa*, can also deliver peptidoglycan into epithelial cells and induce NF- κ B activation. In addition to activating inflammatory pathways, it appears that Nod1 activation can also regulate the secretion of β -defensins, hence protecting against microbial infection.

Another feature of the mucosal innate immune defence against gastrointestinal infections are the mucins, key components of the mucus layer and the epithelial surface. Far from simply providing a barrier for the exclusion of pathogens, it is now clear these complex, highly glycosylated proteins directly interact with host immunity and are themselves important regulators of inflammation. Continuing the *H. pylori* theme, Associate Professor Phil Sutton (Centre for Animal Biotechnology, University of Melbourne, Australia) demonstrated that the cell surface mucin, Muc1, plays an important role in limiting the level of *H. pylori* infection. Muc1 deficiency resulted in elevated *H. pylori* colonisation in mice which was evident from as early as 1 day until at least 2 months post-infection (McGuckin et al., 2007). Moreover, *H. pylori* infection of Muc1-deficient mice induced an atrophic gastritis (the type of

inflammation associated with the risk of development of gastric cancer), in stark contrast to a mild gastritis seen following infection of mice which expressed this mucin. While *H. pylori* were shown to bind this mucin, paradoxically, more bacteria adhered to primary gastric epithelial cells when Muc1 was absent. It was proposed that Muc1 acts as a releasable decoy, to which the bacteria bound and which was then continually shed, removing adherent bacteria from the mucosal surface.

Ghrelin is an immunomodulatory hormone produced by enteroendocrine cells of the gastric mucosa which was recently recognised as possessing anti-inflammatory activity. For example, in mouse models it has been shown to suppress pancreatitis, arthritis and accelerate the healing of gastric ulcers. It can also ameliorate dextran sodium sulfate (DSS) induced colitis. These activities may be due to inhibition of the production of proinflammatory cytokines, possibly by inhibition of NF κ B activation. Multiple peptides with differing activities can be derived from the ghrelin gene. Penny Jeffery investigated the therapeutic activity of these peptides using the 5% DSS model of colitis and found that one, the Δ 4 proghrelin peptide, prevented colon lengthening and weight loss in murine DSS colitis, similar to ghrelin itself, although these anti-inflammatory effects appeared to be limited to the proximal colon. The Δ 4 peptide was demonstrated to significantly increase IL-10 and CCR4 mRNA expression while having no effect on pro-inflammatory cytokines such as IL-1 β and IL-12. In addition, expression of FOXP3 and PPAR γ (essential for Treg mediated protection against colitis) in Tregs and colon extracts respectively was considerably increased by treatment with Δ 4 peptide. Hence the Δ 4 proghrelin peptide appears to have potential for the treatment of colitis, with benefit possibly being derived by the enhancement of mucosal tolerance via Treg activity.

Induction of CD8 T cell responses

Development of effective vaccination strategies for inducing immune responses at mucosal surfaces would be extremely useful for improving protection against a range of mucosal pathogens. One of the more unusual approaches tried is the “inside-out” vaccine pioneered by Professor Andrew Lew (The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia). This involves the injection of a monoclonal antibody against the Mucosal Addressin Cellular Adhesion Molecule-1 (MAdCAM-1) which is expressed on the luminal surface of high endothelial venules and is used as a migration tether for recirculating mucosal lymphocytes. This antibody thus targets mucosal lymphoid tissue and can potentially be used to deliver antigens of choice to these sites. Yifan Zhan (The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia) from the Lew laboratory demonstrated that injection of ovalbumin fused to an anti-MAdCAM-1 antibody induced greater CD8 T cell proliferative responses in mesenteric lymph nodes, compared with injection of the same antigen fused to a non-targeting control antibody (Bourges et al., 2007). Lesser proliferative effects were observed in the spleens and brachial lymph nodes of mice receiving anti-MAdCAM-1 ovalbumin, demonstrating a targeted response in mucosal lymphoid tissues. The induction of these antigen-specific CD8⁺ cells required the presence of dendritic cells as when similar injections were performed in mice depleted of DCs, the CD8 response was considerably ablated. This suggests that ovalbumin delivered to mesenteric lymph nodes by an anti-MAdCAM antibody is cross-presented to CD8 cells by dendritic cells, although the pathway by which the antigen passes from the high endothelial venule surface to dendritic cells contained within the lymph nodes remains to be determined. Importantly, however, these cells had CTL

activity, as demonstrated both by an *in vitro* lysis assay and, most notably, by the induction of protection against subsequent challenge with an OVA expressing tumour.

MUCOSAL VACCINES

The middle ear

The middle ear can be regarded as a mucosal site. It is lined by a mucosa that is a continuum of that of the nasopharynx and responds immunologically similarly to that observed at other mucosal sites (Kurono & Mogi, 1996). Infective agents track into the normally middle ear space via the Eustachian tube to cause infection. The major bacterial pathogens that cause OM are *S. pneumoniae*, NTHi and *M. catarrhalis* and there is a correlation between colonisation and concurrent infection with these microbes (Faden et al 1990). There is a paucity of information concerning the immunology of the middle ear and response to infection at this site and further studies are required. Professor Jennelle Kyd (Central Queensland University, Australia) reviewed the preclinical animal model data which indicate that vaccination is a feasible intervention to prevent infection of the middle ear. A number of vaccine antigens have been identified and require human trials (Kyd et al 2003; Cripps et al 2005; Cripps & Otczyk 2006).

Vaccination against influenzae H5N1

The pathogenesis of H5N1 influenzae and potential risk of a major influenzae pandemic was reviewed by Dr Ian Barr (CSL Ltd, Victoria, Australia). There is no pre-existing humoral immunity to H5 in the human population. The major reasons for the severity of disease following infection include the overwhelming inflammatory host response and ability of the virus to rapidly replicate to high titres. Three fundamental control measures exist for H5N1: vaccination, antiviral drugs such as M2

ion channel inhibitors as well as neuraminidase inhibitors and finally a series of public health measures. The ferret remains the best animal model to test influenza vaccines as it is susceptible to all infecting human types. Studies presented demonstrated that immunization of ferrets with H5N1 test formulations induced good protection against subsequent challenge. Whilst there is good correlation between the positive results observed in the animal studies and early human trials, H5N1 appears to be poorly immunogenic in humans, requiring more than one vaccination with adjuvant. In conclusion the speaker spoke of the positive collaboration that now exists between researchers, industry and regulatory authorities to focus on the development of measures to use against the threat of a global influenza outbreak.

Vaccine delivery systems

A number of adjuvant systems have been tested experimentally for their potential use to enhance mucosal immunity in humans. Unfortunately many of these have unwanted toxic or adverse side effects. Danica Hickey (Immunology & Microbiology, University of Newcastle, Australia) presented her findings on a lipid transdermal formulation – LipoVax (Aldwell et al 2003). LipoVax was formulated with the major outer membrane protein (MOMP) of *Chlamydia muridarum* and compared against a formulation containing CpG/cholera toxin for protection against respiratory or genital infection by *C. muridarum*. The LipoVax transdermal-MOMP formulation was as effective as the CpG/CT-MOMP formulation in protecting against respiratory challenge and genital challenge with *C. muridarum*. The LipoVax-MOMP formulation appeared to induce greater IgA responses in the lavages of both the respiratory and genital tracts. Further studies are warranted on the use of transdermal delivery systems to induce protective mucosal immune responses.

Recently there have been a number of reports suggesting that it may be possible to specifically target innate M cell receptors of the Peyer's patches to enhance mucosal immune responses (Foxwell et al 2007). Yok Teng Chionh (Centre for Animal Biotechnology, University of Melbourne, Australia) presented the concept of targeting M cell receptors with *Ulex europaeus* 1 (UEA-1) lectin agglutinated *H. pylori*. This technology has previously been explored to target PLGA nanoparticles (Gupta et al 2006). Following oral immunization of mice with agglutinated bacteria, significantly raised levels of both IgG and IgA specific *H. pylori* antibody was observed in serum, intestinal mucous and vaginal washings. However, accelerated clearance of *H. pylori* infection was not observed. The studies were extended to *Campylobacter jejuni* and a similar enhancement of serum and intestinal IgG and IgA specific *C. jejuni* antibodies to that observed for *H. pylori* was observed. However, in contrast to *H. pylori* an enhanced clearance of the *C. jejuni* infection was observed. The specific targeting of vaccines to M cells is a promising strategy for mucosal immunisation. Further research is required using lectins such as UEA-1 to optimise this approach and eventually to undertake human studies.

CONCLUDING REMARKS

Australia has over the last century made a significant contribution to immunology and infectious diseases with three of her citizens being awarded a Nobel Prize for their outstanding and innovative research. Australian scientists have also made major contributions, over again a long period of time, to our understanding of the interaction between the mucosal immune surface and infective and environmental as well as reproductive events. This research has had widespread applications to both human health and the veterinary industry. This Workshop report provides a very brief overview of some of the mucosal immunology research that is currently being

undertaken in 12 organisations across Australia. Areas of research included the respiratory tract and middle ear, the reproductive tract and the gastrointestinal tract. Presentations also covered fundamental immunological mechanistic studies and vaccine applications. The presentations were given by 2 internationally renowned mucosal immunologists, leading Australian mucosal immunologists and most importantly a number of early career researchers. Mucosal immunology down-under remains an area of great research activity a decade after 9-ICMI.

ACKNOWLEDGEMENTS

Support of the ASI and the SMI is gratefully acknowledged. A special thanks to all of those who attended the workshop, and those who presented (Ian Barr, Ken Beagley, Alison Carey, Yok Teng Chionh, Margaret Dunkley, Richard Ferrero, Mark Hedger, Danica Hickey, Penny Jeffery, Ajay Krishnamurthy, Jennelle Kyd, Reinhard Pabst, Sarah Robertson, Phil Sutton, Matthew Wikstrom, Bruce Wines, Charles Wira, Amanda Yeung, Yifan Zhan).

REFERENCES

- Aldwell FE, Tucker IG, de Lisle GW, Buddle BM. Oral delivery of *Mycobacterium bovis* BCG in a lipid formulation induces resistance to pulmonary tuberculosis in mice. *Infect. Immun.* 2003; 71: 101-108.
- Berry LJ, Hickey DK, Skelding KA, Bao S, Rendina AM, Hansbro PM et al. Transcutaneous immunization with combined cholera toxin and CpG adjuvant protects against *Chlamydia muridarum* genital tract infection. *Infect. Immun.* 2004; 72: 1019-1028.
- Blum KS, Pabst R. Lymphocyte numbers and subsets in the human blood. Do they mirror the situation in all organs? *Immun. Lett.* 2007; 108: 45-51.
- Bourges D, Zhan Y, Brady JL, Braley H, Caminschi I, Prato S et al. Targeting the gut vascular endothelium induces gut effector CD8 T cell responses via cross-presentation by dendritic cells. *J. Immunol.* 2007; 179: 5678-5685.
- Brandtzaeg P, Kiyono H, Pabst R, Russell MW. Terminology: nomenclature of mucosal-associated lymphoid tissue. *Mucosal Immunol.* 2008; 1: 31-7.
- Cripps AW, Otczyk DC. Prospects for a vaccine against otitis media. *Expert Rev. Vaccines* 2006; 5: 517-34.
- Cripps AW, Otczyk DC, Kyd JM. Bacterial otitis media: a vaccine preventable disease? *Vaccine* 2005; 23: 2304-2310.
- Faden H, Stanievich J, Brodsky L, Bernstein J, Ogra PL. Changes in nasopharyngeal flora during otitis media of childhood. *Pediatr. Infect. Dis. J.* 1990; 9: 623-626.
- Foxwell AR, Cripps AW, Kyd JM. Optimization of oral immunization through receptor-mediated targeting of M cells. *Human Vaccine* 2007; 3: 220-223.

- Girling JE, Hedger MP. Toll-like receptors in the gonads and reproductive tract: emerging roles in reproductive physiology and pathology. *Immunol. Cell Biol.* 2007; 85: 481-9.
- Gupta PN, Mahor S, Rawat A, Khatri K, Goyal A, Vyas SP. Lectin anchored stabilized biodegradable nanoparticles for oral immunization: 1. Development and *in vitro* evaluation. *Inter. J. Pharm.* 2006; 318: 163-173.
- Kaparakis M, Philpott DJ, Ferrero RL. Mammalian NLR proteins; discriminating foe from friend. *Immunol. Cell Biol.* 2007; 85: 495-502.
- Kurono Y, Mogi G. Mucosal immunity of the middle ear. In *Mucosal vaccines*. Kiyono J, Ogra PL, McGhee J (eds) California, USA: Academic Press, 1996, pp 451-458.
- Kyd JM, Cripps AW, Novotny AL, Bakaletz LO. Efficacy of the 26-kilodalton outer membrane protein and two P5 fimbrin-derived immunogens to induce clearance of nontypeable *Haemophilus influenzae* from the rat middle ear and lungs as well as from the chinchilla middle ear and nasopharynx. *Infect. Immun.* 2003; 71: 4691-9.
- Luhrmann A, Grote K, Stephan M, Tschernig, T, Pabst R. Local pulmonary immune stimulation by the Toll-like receptor 2 and 6 ligand MALP-2 in rats is age dependent. *Immunol. Lett.* 2007; 108: 167-173.
- McGuckin MA, Every AL, Skene CD, Linden SK, Chionh YT, Swierczak A et al. Muc1 mucin limits both *Helicobacter pylori* colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology* 2007; 133: 1210-1218.
- Ramsland PA, Willoughby N, Trist HM, Farrugia W, Hogarth PM, Fraser J. Structural basis for evasion of IgA immunity by *Staphylococcus aureus* revealed in the complex of SSL7 with Fc of human IgA1. *PNAS* 2007; 104: 15051-15056.

- Robertson SA. Seminal plasma and male factor signalling in the female reproductive tract. *Cell Tissue Res.* 2005; 322: 43-52.
- Sharkey DJ, Macpherson AM, Tremellen KP, Robertson SA. Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells. *Mol. Hum. Reprod.* 2007; 13: 491-501
- Skelding KA, Hickey DK, Horvat JC, Bao S, Roberts KG, Finnie JM et al. Comparison of intranasal and transcutaneous immunization for induction of protective immunity against *Chlamydia muridarum* respiratory tract infection. *Vaccine* 2006; 24: 355-66.
- Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP et al. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. *Nat. Immunol.* 2004; 5: 1166-1174.
- von Garnier C, Wiikstrom ME, Zosky G, Turner DJ, Sly PD, Smith M et al. Allergic airways disease develops after an increase in allergen capture and processing in the airway mucosa. *J. Immunol.* 2007; 179: 5748-5759.