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Linfa Wang
Duke-NUS Medical School, Singapore, SINGAPORE
Emerging bat viruses in the context of the COVID-19 pandemic

Leo Poon
University of Hong Kong, Hong Kong, HONG KONG
SARS-CoV-2 and its transmission

Keith Chappell
University of Queensland, St Lucia, QLD, Australia
Molecular clamp stabilized subunit vaccine for COVID-19

Julian Hiscox
University of Liverpool, Liverpool, MERSEYSIDE, United Kingdom
Analysis of SARS-CoV-2 in clinical samples, post-mortem tissue and animal models and what this tells us about COVID-19 in severe cases

Moritz Kramer
Harvard University, USA, United States
The effect of human mobility and population structure on COVID-19 epidemics

Erica Ollmann-Saphire
La Jolla Institute for Immunology, California, United States
Antibodies against Emerging Infectious Diseases - global collaborations

Burkhard Becher
University of Zurich Switzerland, Zurich, Switzerland
Single cell mapping of human brain cancer reveals tumor-driven education education of tumor-associated leukocytes

I-hsin Su
Nanyang Technological University Singapore, Singapore, SINGAPORE
Talin1 sets the stage for TLR-mediated activation of dendritic cells

Mariapia Degli-Esposti
Monash University, Clayton, VIC, Australia
Insights into the requirements for long-term cytomegalovirus immunity
Cheng-I Wang, Sandy Lee  
*Singapore Immunology Network, A*STAR, Singapore*  
Effective killing of acute myeloid leukemia by TIM-3 targeted chimeric antigen receptor T cells

Katie Owen  
*Latrobe Institute For Molecular Science, Bundoora, VIC, Australia*  
Enhancing tumor-intrinsic signaling to prevent the metastatic spread of cancer cells to bone

Siok Tey  
*QIMR Berghofer Medical Research Institute, Heston, QLD, Australia*  
Phase I clinical trial using gene-modified T cells within an academic setting

Toby Lawrence  
*Kings College London, London, United Kingdom*  
Targeting Tumour-associated Macrophages

Paul Macary  
*National University of Singapore, Singapore*  
Developing antibody-based tools for the diagnosis and treatment of Dengue

Junyun Lai  
*Peter MacCallum Cancer Centre, Melbourne, VICTORIA, Australia*  
Overcoming tumour heterogeneity by engaging host immunity to enhance chimeric antigen receptor (CAR) T cell therapy of solid cancers

Katherine Kedzierska  
*University of Melbourne, Parkville, VIC, Australia*  
Understanding immunity to SARS-CoV2 infection

Lisa Ng  
*Singapore Immunology Network, A*STAR, Biopolis, SINGAPORE, Singapore*  
Cellular and molecular mechanisms of viral infections: from diagnostics to therapies

Antonio Bertoletti  
*Duke - NUS, Singapore*  
T cell response against SARS-CoV2
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9th November 2020

Welcome and Official Opening
10:50AM - 11:00AM
Chair: Ian Barr

Session 1: COVID-19
11:00AM - 12:00PM
Chairs: Lisa Ng & Ian Barr

Session supported by

11:00 AM Linfa Wang
Emerging bat viruses in the context of the COVID-19 pandemic abs# 1

11:17 AM Leo Poon
SARS-CoV-2 and its transmission abs# 2

11:36 AM Keith Chappell
Molecular clamp stabilized subunit vaccine for COVID-19 abs# 3

Break
12:00PM - 12:10PM

Session 2: Emerging Diseases
12:10PM - 1:10PM
Chairs: Laurent RENIA & Gabrielle Belz

12:10 PM Julian Hiscox
Analysis of SARS-CoV-2 in clinical samples, post-mortem tissue and animal models and what this tells us about COVID-19 in severe cases abs# 4

12:27 PM Moritz Kramer
The effect of human mobility and population structure on COVID-19 epidemics. abs# 5
12:34 PM Erica Ollmann-Saphire
Antibodies against Emerging Infectious Diseases - global collaborations abs# 6

Break
1:10PM - 1:25PM

Session 3: Innate Immunity
1:25PM - 2:25PM
Chair: Jose Villadangos

1:25 PM Burkhard Becher
Single cell mapping of human brain cancer reveals tumor-driven education of tumor-associated leukocytes abs# 7

1:42 PM I-hsin Su
Talin1 sets the stage for TLR-mediated activation of dendritic cells abs# 8

2:02 PM Mariapia Degli-Esposti
Insights into the requirements for long-term cytomegalovirus immunity abs# 9

Break
2:25PM - 2:35PM

Session 4: Virtual Poster Presentations
2:35PM - 3:25PM
Chairs: Jose Villadangos & Laurent Renia

2:35 PM Ian H Frazer
Polynucleotide immunotherapy for HPV associated oropharyngeal cancer abs# 101

2:40 PM Mariusz Skwarczynski
Polymerized Amino Acids as a Self-Adjuvanting Delivery System for Vaccine against Group A Streptococcus abs# 102

2:45 PM Mohammad Omer Faruck
Development an oral-delivery system for peptide vaccine against Group A Streptococcus abs# 103
2:50 PM Farrhana Firdaus  
Development of Lipopeptide-based Adjuvants as Vaccine Candidates against Group A Streptococcus (GAS) abs# 104

2:55 PM Thi HO Nguyen  
Integrated immune dynamics define correlates of COVID-19 severity and antibody responses abs# 105

3:00 PM Yi-Hao Chan  
Immune correlates of COVID-19 severity and modulation with cyclooxygenase-2 inhibitors abs# 106

3:05 PM Siew-Wai Fong  
Immune landscape of 382-nt deleted SARS-CoV-2 reveals heightened adaptive response indicating prophylactic potential against COVID-19 abs# 107

3:10 PM Chek Meng Poh  
Two linear B cell epitopes on SARS-CoV-2 spike protein elicit neutralizing antibodies in COVID-19 patients abs# 108

3:15 PM Stephanie Gras  
An investigation of the T cell response against viruses through a structural lens abs# 109
10th November 2020

Session 5: Immunotherapies
11:00AM - 12:00PM

Chairs: Phil Darcy & Rajiv Khanna

Session supported by

11:00 AM Sandy Lee
Effective killing of acute myeloid leukemia by TIM-3 targeted chimeric antigen receptor T cells abs# 10

11:20 AM Katie Owen
Enhancing tumor-intrinsic signaling to prevent the metastatic spread of cancer cells to bone abs# 11

11:34 AM Siok Tey
Phase I clinical trial using gene-modified T cells within an academic setting abs# 12

Break
12:00PM - 12:10PM

Session 6: Inflammation/Anitbody Therapies
12:10PM - 1:10PM

Chairs: Subhra k Biswas & Christina Scheffler

12:10 PM Toby Lawrence
Targeting Tumour-associated Macrophages abs# 13

12:27 PM Paul Macary
Developing antibody-based tools for the diagnosis and treatment of Dengue abs# 14

12:44 PM Junyun Lai
Overcoming tumour heterogeneity by engaging host immunity to enhance chimeric antigen receptor (CAR) T cell therapy of solid cancers abs# 15

Break
1:10PM - 1:25PM
Session 7: Viral Immunity
1:25PM - 2:25PM

Chairs: Rosemary Ffrench & Junyun Lai

Session supported by

1:25 PM Katherine Kedzierska
Understanding immunity to SARS-CoV2 infection abs# 16

1:47 PM Lisa Ng
Cellular and molecular mechanisms of viral infections: from diagnostics to therapies abs# 17

2:05 PM Antonio Bertoletti
T cell response against SARS-CoV2 abs# 18

Break
2:25PM - 2:35PM

Session 8: Virtual Poster Presentations
2:35PM - 3:25PM

Chairs: Gabrielle Belz & Rosemary Ffrench

2:35 PM John Miles
Using synthetic biology to generate hyper-stable vaccines abs# 201

2:40 PM Denise Doolan
Effective targets of cross-species protective immunity against malaria identified by proteome-wide screening abs# 202

2:45 PM Christina Scheffler
Rapid inactivation and deletion of adoptively transferred CTL upon encounter with a high number of antigen presenting lymphoma cells abs# 203

2:50 PM Paul J Neeson
Stem cell memory like CAR-T cells persist better in vivo and induce solid tumour complete regression in combination with anti-PD1. abs# 204
2:55 PM **Rong En Tay**  
Hdac3 is an epigenetic inhibitor of the cytotoxicity program in CD8 T cells *abs# 205*

3:00 PM **So Young Chang**  
TCR repertoire and transcriptome differs between optimal HLA-A*02:01- and high-risk HLA-A*24:02-restricted CD8+ T cell immunity against influenza A virus *abs# 206*

3:05 PM **Hui Jing Lim**  
The discovery of molecular regulatory mechanism of MR1 antigen presentation *abs# 207*

3:10 PM **Diana H Quan**  
Optimising novel adjuvant formulations to promote protective immunity without reactogenicity *abs# 208*

3:15 PM **Erica Stewart**  
Intrapulmonary vaccination with delta-inulin adjuvant stimulates non-polarised chemotactic signalling and diverse cellular interaction *abs# 209*
Overcoming tumour heterogeneity by engaging host immunity to enhance chimeric antigen receptor (CAR) T cell therapy of solid cancers

Junyun Lai1, Sherly Mardiana1, Imran House1, Kevin Sek1, Melissa Henderson1, Lauren Giuffrida1, Amanda Chen1, Kirsten Todd1, Emma Petley1, Jack Chan1, Emma Carrington2, Andrew Lew2, Benjamin Solomon3, Joseph Trapani3, Katherine Kedzierska4, Maximilien Evrard3, Stephan Verwoert3, Jason Waithman4, Phillip Darcy4, Paul Beavis5

1. Peter MacCallum Cancer Centre, Melbourne, VICTORIA, Australia
2. The Walter and Eliza Hall Institute of Medical Research, Melbourne, VICTORIA, Australia
3. The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, VICTORIA, Australia
4. Telethon Kids Institute, Perth, WA, Australia

Adoptive cell therapy (ACT) using chimeric antigen receptor (CAR) T cells is a form of immunotherapy where T cells are genetically modified to recognise tumour antigens and specifically target cancer. While CAR-T cells have achieved remarkable therapeutic efficacy for some blood cancers, its effect in solid cancers has remained limited. One confounding issue is the variable expression of target antigens on solid tumours (i.e. tumour heterogeneity). CAR-T cells may eliminate antigen-expressing but not antigen-negative tumour cells, which can consequently lead to disease relapse involving the latter. Dendritic cells (DCs) are professional antigen-presenting cells specialised in the priming and activation of T cells. We hypothesised that engaging the host immune system by enhancing DCs will improve host T cell anti-tumour responses and overcome tumour heterogeneity in ACT. To this end, we engineered T cells to secrete DC growth factor Fms-like tyrosine kinase 3 ligand (FL). Mice treated with FL-secreting T cells showed expanded host DC and T cell numbers in tumours. Combination of FL-secreting T cells with immune-stimulatory adjuvants further inhibited tumour growth in models of ACT and CAR-T cell therapy in a host DC and T cell-dependent manner. Importantly, combination therapy was associated with a significant increase in host anti-tumour T cells recognising antigens beyond those targeted by the CAR (epitope spreading). Our data suggest that enhancing host anti-tumour immunity represents a promising strategy to improve the overall efficacy of CAR-T cell therapy against solid tumour heterogeneity, which may help combat the clinical problem of antigen-negative tumour relapse following therapy.

Polynucleotide immunotherapy for HPV associated oropharyngeal cancer

Ian H Frazer1, Janin Chandra1, Neil Finlayson2, Howard Liu3, Rahul Ladwa1, Yvonne Woo1, Yan Xu1, Sandro V Porceddu1

1. Faculty of Medicine, University of Queensland, Brisbane, Queensland, Australia
2. Jingang Medicine (Australia) Pty Ltd, Brisbane, QLD, Australia
3. Princess Alexandra Hospital, Brisbane, QLD, Australia

Background: HPV associated oropharyngeal cancers (OPC) are increasing in prevalence worldwide. Treatment of disease recurrence, either locoregionally or distant, after primary chemoradiotherapy or surgery is not uncommon, and poses a difficult management dilemma with limited success. Polynucleotide vaccines encoding HPV16 E6/E7 fusion proteins have demonstrated efficacy in several animal models of HPV associated cancer.

Aim: Evaluate a polynucleotide immunotherapy targeted at HPV16 E6 and E7 proteins for immunogenicity and safety in patients with apparent cure after primary therapy for HPV associated OPC.

Methods: A 1:1 mixture of 2 codon modified polynucleotide vaccines encoding HPV16 E6 and E7 with or without ubiquitin were administered at three doses (0.25mg, 1mg, 4mg) intracutaneously on 3 occasions, 4 weekly, to a total of 12 subjects with treated HPV associated OPC.

Results: A cell mediated response to HPV 16 E6 and E7 was evident at baseline in all participants using ELISpot. Antibodies against HPV 16 E7 was evident at baseline in 11 of 12 participants using ELISA. Of 12 subjects, 10 demonstrated a significant immune response to one or more of the peptide pools at one or more timepoints. One subject has had a confirmed recurrence of disease 6 months after immunisation. Only minor local adverse events attributable to the vaccine at the site of injection were observed.

Conclusion: This polynucleotide vaccine enhanced specific immunity to a virus derived tumour associated antigen in the majority of immunised subjects without significant adverse events, warranting a further study in subjects with recurrent disease after treatment.

This study was conducted with the approval of the Australian Therapeutic Goods Administration and the Ethics committee of the Princess Alexandra Hospital.
Polymerized Amino Acids as a Self-Adjuvanting Delivery System for Vaccine against Group A Streptococcus

Mariusz Skwarczynski¹, Istvan Toth¹, 2, 3

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Introduction
Vaccines based on the whole pathogen as well as protein-based vaccines are able to deliver protection against variety of pathogens; however, they are often not fully safe and may induce undesirable immune responses (e.g., inflammation, allergy, etc) [1]. Moreover some vaccines must use even smaller component than protein to avoid triggering autoimmune responses, for example all modern vaccines against Group A Streptococcus (GAS) that have entered clinical are based on peptide antigens [2]. Thus, peptide-based vaccine can solve above problems; however, they need powerfully adjuvants (immunostimulators), which are usually bacteria derived and are associated with some toxicity [3, 4].

Previously we have demonstrated that hydrophobic dendritic poly(tert-butyl acrylate) can be conjugated to a variety of peptides epitopes, including GAS-derived peptides, self-assembled to form particles, which can induce potent humoral and cellular immune responses [5-7]. However, these polymers were not biodegradable, had undefined stereochemistry and number of unit repeats. This typical variability of polymer structure may affect in vitro and in vivo efficacy of vaccine and therefore might not be suitable for clinical trials.

Thus we have designed fully-defined and biodegradable polymers compose of natural hydrophobic amino acids (HAA) [8]. Polymer produced based on this system are fully defined and biodegradable to non-toxic natural amino acids. For purpose of this study we selected conserved B-cell epitope derived from GAS M-protein (J8, QAEDKVKQSQREAKKQVEKALKQLEDKVQ), and a universal T-helper epitope (PADRE, AKFVAWTLKAAA) which were incorporated into pHAA-based system (see Figure 1).

Materials and methods
All the vaccine candidates were synthesized by Boc-solid-phase peptide synthesis (SPPS) method. Particle following simple self-assembly in water/PBS were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Their secondary structure were measured by circular dichroism (CD). Cytotoxicity against SW620 (human colon carcinoma) and HEK293 (human embryonic kidney) were examined MTT assay. Antigen presenting cells (APCs) maturation was examined in spleen cells isolated from naïve C57BL/6 mice using CD40 and MHCII markers. Vaccination was performed subcutaneously in C57BL/6 female mice. The mice were challenged intranasally with the M1 GAS strain one month after immunization. ELISA assay was used to measure antibody titers. An opsonization (bactericidal) assay was performed with immunized mice serum against variety of clinical GAS isolates.

Results
The pHAA, J8 and PADRE were conjugated on the resin using the standard Boc-SPPS method. Produced conjugates were self-assemble in PBS to form chain-like aggregates of the nanoparticles (CLAN) as determined was visible on TEM images, with each nanoparticle size around 20 nm. Conjugate bearing polyleucine adopted helical conformation required for J8 epitope to generated conformation antibody against GAS M protein. All the examined conjugate were non-toxic to human cells lines and not adverse effects during in vivo study was observed. Polyleucine conjugate was the most efficient in stimulation of APCs maturation. The endotoxin level in this formulation was negligible. All conjugates elicited significant J8-specific IgG titers after the final immunization. Polyleucine conjugate induced significantly stronger responses than complete Freund’s adjuvant (CFA), and all other conjugates, in both serum and saliva. Antibodies produced by mice immunized with the conjugated were able to opsonize all tested GAS clinical isolates. Furthermore, only polyleucine-based conjugate greatly reduce bacteria burden in Nasal Associated Lymphoid Tissue (NALT; a murine functional homolog to human tonsils) and the spleen, as well as in nasal shedding, and throat swabs. The powerful but toxic CFA adjuvant was less effective. Moreover, the conjugate did not induce inflammatory responses, which are often related to undesired side effects when classical adjuvants are incorporated in the vaccines.

Conclusions
The discovery of potent and safe self-adjuvanting delivery system for poorly immunogenic antigens is one of the major challenges in vaccine development. Here we demonstrated that conjugation of peptide antigen to polymerized hydrophobic amino acids can form nanoparticles, which can induce strong humoral immune responses. This new polymeric system is fully-defined, has no chain or stereochemistry variability, and biodegradable to non-toxic natural amino acids. It can be customized to adopt the polymer to selected antigen, for example to produce nanoparticle of desired size. Therefore, the system is expected to find application in the vast variety of vaccines against infectious diseases.

Acknowledgements
We thank the facilities, and the scientific and technical assistance, of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy and Microanalysis, The University of Queensland. We also acknowledge AD Paterson and P Harris (The University of Queensland Centre for Clinical Research) for providing Streptococcus isolates, GC 2 203, D3840 and D2612. This work was supported by the National Health and Medical Research Council [NHMRC Program Grant APP1132975 and NHMRC Project Grant APP1099999]. We also thank Guangzu Zhao, Jennifer C. Boer, Ashwini Kumar Giddam, Manisha Pandey, Mohini A. Shibu, Reshma J. Nevagi, Michael R. Batzloff, James W. Wells, Robert J. Capon, Victoria Ozberk; Armira Azuar; Jazmina Gonzalez Cruz; Zeinab G. Khalil, Waleed M. Hussein, Magdalena Plebanski, Michael F. Good for their contributions in this work.

References

Development of an oral-delivery system for peptide vaccine against Group A Streptococcus

Mohammad Omer Faruck1, Mariusz Skwarczynski1, Istvan Toth1
1. School of chemistry and molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia

Group A Streptococcus or GAS infection are responsible for over 1.4 million deaths each year, and this number is still increasing. Vaccination is considered as a useful approach to enhance the host immunity against infection, and it has helped to prevent and even eradicate many infectious diseases so far. Herein we developed a potent peptide-polymer based vaccine against GAS infection. Our fully synthetic peptide vaccine candidates against group A streptococcus (GAS) were composed of J8 GAS B-cell epitope alongside with a universal helper T-cell epitope PADRE. Alkyn based peptide (J8-PADRE) was conjugated with azide based polymer named poly methyl acrylate (PMA) by Copper-Catalyzed Alkyn-Azide Cycloaddition (CuAAC). PMA-P-J8 formed nanoparticle size (146±8 nm) and PDI (0.19±0.02) measured using dynamic light scattering (DLS). PMA is one of the most widely explored bio-medical polymers because of its biocompatibility. Fourteen weeks old female mice (C57BL/6) were immunized with PMA-P-J8 and positive control with additional cholera toxin B (CTB) by oral gavage. PBS was used as negative control with a single oral immunization. All groups of mice that received GAS vaccine developed anti-GAS antibodies as determined by ELISA. The addition of CTB did not result in greater anti-GAS antibody titres. In fact, mice immunized without CTB had greater anti-GAS antibody titres than mice immunized with CTB because CTB can lower the immune response to oral vaccination. The antibodies generated were opsonic against GAS clinical isolates as measured after boost immunization. Thus, we developed a simple conjugate as an effective, adjuvant-free oral peptide-based vaccine. The enhanced vaccine efficacy, lowered dose, and simple and cost-effective process should be particularly useful in developing potent peptide-based vaccines to prevent infection.

Development of Lipopeptide-based Adjuvants as Vaccine Candidates against Group A Streptococcus (GAS)

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Vaccination is the most efficient medical invention to counter the effects of infectious diseases to date. Overtime, vaccine designs have evolved into more precisely defined formulations wuth antigens. Peptide-based subunit vaccines utilises selected microbial fragments which can eliminate adverse effects related issues associated with conventional vaccines [1]. However, peptide epitopes are known to be poor immunogens and would require the use of an adjuvant to enhance vaccine immunogenicity [2]. The addition of lipid moieties to the epitopes can improve the efficacy of peptide-based vaccines [3]. The main objective of the study was to evaluate and optimise the ability of lipopeptides (lipid-core peptides, antimicrobial peptides and short cationic peptides) to trigger immune response against the chosen antigens (J8, B-cell epitope derived from Group A Streptococcus (GAS) and universal T helper (P25)). The vaccine candidates was analysed as a mixture (physical mixture of lipopeptide and antigens) and conjugated construct (antigens conjugated to lipopeptide). A series of potential lipopeptide-based adjuvants, conjugated construct and antigens were synthesised through solid phase peptide synthesis and analysed with mass spectrometry and reverse phase high performance liquid chromatography. Vaccine candidates were characterized with dynamic light scattering and circular dichroism. Additionally, enzyme-linked immunosorbent assay was used to detect and quantify J8-specific IgG titers collected from immunological studies performed in mice. Through the characterisation of the compounds, it was discovered that the particle size of the mixture was more stable and homogenous, suggesting the formation of complexes. Additionally, the secondary structure of the mixture was found to be a combination of α-helical and random coil similar to the conjugated construct. In vivo assessment of the vaccine candidates showed the conjugated construct was more effective. Except, one mixture (counterpart to the conjugated construct consisting of lipid-core peptides) was able to induce higher J8-specific IgG antibodies than the conjugated construct. We were able to identify one lipopeptide with promising adjuvanting abilities which elicited higher IgG titers against the antigen when conjugated and in a mixture with the antigen in comparison to the other lipopeptides.
Integrated immune dynamics define correlates of COVID-19 severity and antibody responses

Marios Koutsakos¹, Louise C Rowntree¹, Luca Hensen¹, Brendon Y Chua¹,², Carolien E van de Sandt¹,³, Jennifer R Habel¹, Wuji Zhang¹, Xiaoiaojia Li¹, Lukasz Kedzierski¹,², Thomas M Ashhurst⁴,⁵, Givana H Putri⁶,⁷, Felix Marsh-Wakefield⁸,⁹,¹⁰, Mark N Read¹¹,¹², Davis N Edwards¹³,¹⁴, Bridie Clemens¹, Chiin Yi Wong¹, Francesca L Mordant¹, Jennifer A Juno¹, Fatima Amanat¹⁴,¹⁵, Jennifer Audsley¹⁶, Natasha E Holmes¹⁷,¹⁸, Carly M Hughes¹⁹, Mike Cattion²⁰, Justin Denholm²¹,²², Steven YC Tong²³,²⁴, Denise L Doolan²⁵, Tom C Kotsimbos²⁶,²⁷, David C Jackson²⁸, Florian Krammer²⁹, Dale I Godfrey³⁰, Amy W Chung³¹, Nicholas JC King³²,³³,³⁴, Sharon R Lewin³⁴,³⁵,³⁶, Irani Thevarajan³⁷,³⁸, Allen C Cheng³⁹,⁴⁰, Katherine Kedzierska¹,², Thi HO Nguyen¹

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Abstract:
SARS-CoV-2 causes a spectrum of illness, ranging from asymptomatic to severe COVID-19. Early in March when WHO declared the COVID-19 pandemic, we provided the first evidence that the breadth of robust immune responses to SARS-CoV2 can be measured in peripheral blood prior to patient recovery. This was a longitudinal case study of one patient. As the immunological
basis for severity remains ill-defined, we have now analysed 78 SARS-CoV-2-infected individuals at acute and/or convalescent timepoints, up to 102 days post-symptom onset, quantifying 184 innate and adaptive immunological parameters. Acute hospitalised COVID-19, including ward and intensive care unit (ICU) patients, was associated with high levels of IL-6, IL-18 and IL-10, elevated neutrophil-to-lymphocyte and neutrophil-to-T cell ratios, and high proportions of activated CD38+ neutrophils, CD38+ eosinophils, CD38+HLA-DR+ monocytes, CD38+CD56+ NK cells, CD38+γδ T cells, antibody-secreting cells, PD-1+ICOS+ circulating T follicular helper (Tfh) cells, CD38+HLA-DR+CD4+ T cells, effector CD27+CD45RA+ and CD38+CD8+ T cells. During convalescence, elevated seroconversion and neutralising antibodies were prominent and correlated with acute CXCR3+ Tfh cell activation. Strikingly, ICU patients with severe COVID-19 displayed higher levels of soluble IL-6R, IL-18, and hyperactivation of innate, adaptive and myeloid compartments than ward patients with moderate disease. Our analyses provide a comprehensive map of longitudinal immunological responses in COVID-19 patients during acute and convalescent phases of SARS-CoV-2 infection, and integrate key cellular pathways of complex perturbed immune networks that underpin severe COVID-19. We observed a typical immune response in people displaying mild to moderate severity. IL6, IP10, elevated neutrophil and immunotherapies for severer cases such as higher IL-8, IL-6R, IL-6 and CD38 expression on innate and adaptive immune cells.

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**Immune correlates of COVID-19 severity and modulation with cyclooxygenase-2 inhibitors**

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a highly transmittable pathogen that is responsible for the pandemic of acute respiratory disease, termed Coronavirus Disease 2019 (COVID-19). This pathogen has a huge impact on public health, and international efforts are underway to decipher the underlying immune responses orchestrating the interplay between protection and inflammation. However, key gaps remain, which can have implications on prognosis and therapeutic strategies against COVID-19. In a prospective observational cohort study of 81 patients, we measured plasma samples for levels of cytokines and chemokines and found that levels of pro-inflammatory IL-6, IP-10, IL-12, and IL-18 correlated with disease severity. IL-6 has also been identified as a key cytokine in the inflammatory cascade in other studies. This suggests that immunomodulators may be beneficial as an adjunct or alternative to antivirals. Consequently, a small-scale trial with COX-2 inhibitors was performed, with promising reduction of IL-6 in high-risk COVID-19 pneumonia patients more than 50 years of age. Treatment was also not associated with an increase in adverse outcomes. Its potential for therapeutic use as an immune modulator warrant further evaluation in a large randomized controlled trial.

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**Immune landscape of 382-nt deleted SARS-CoV-2 reveals heightened adaptive response indicating prophylactic potential against COVID-19**

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Publish consent withheld
Two linear B cell epitopes on SARS-CoV-2 spike protein elicit neutralizing antibodies in COVID-19 patients

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COVID-19, caused by SARS-CoV-2 coronavirus, has become a grave threat to global public health in 2020, causing over a million deaths and prompting governments worldwide to enter lockdowns to control the pandemic. The SARS-CoV-2 spike protein acts as the ligand to bind with human ACE2 to mediate viral entry into host cells. Thus, specific antibodies that interfere with this process are useful in halting the infection. To identify SARS-CoV-2 specific epitopes, we collected the sera of 25 convalescent patients during the first wave of COVID-19 infection in Singapore and 13 donors who recovered from SARS-CoV (SARS) infection in 2003 for analysis. Sera from COVID-19 patients, but not recalled SARS patients, contain antibodies that neutralize pseudotype lentivirus expressing the SARS-CoV-2 spike protein. Furthermore, we screened these sera against the peptide libraries spanning the spike protein of SARS-CoV-2 and uncovered two specific linear B cell epitopes, S14P5 and S21P2, that were recognized only by COVID-19 patient sera. S14P5 is situated close to the receptor-binding domain (RBD) while S21P2 overlaps with the fusion peptide sequence, suggesting that specific antibodies against these regions may be able to interfere with viral entry into host cells. Indeed, depleting antibodies that recognize S14P5 and S21P2 decreased neutralization capacity of sera against pseudotype lentivirus expressing the SARS-CoV-2 spike protein. In conclusion, antibodies against S14P5 and S21P2 can interfere with viral entry into host cells and prevent infection, indicating that they have considerable therapeutic potential to be used in COVID-19 treatments.

An investigation of the T cell response against viruses through a structural lens

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T cells are a critical part of the immune response, that would determine the fate of an infection and disease outcome. Our Lab is focused on understanding how T cell engage with viral particles, called peptide antigens, that are presented by highly polymorphic molecules called Human Leukocyte Antigens (HLA). T cells have receptors on their surface called T cell receptor (TCR) that allow them to recognise the composite surface of the peptide-HLA complex.

Using X-ray crystallography we are seeking to understand both peptide antigens presentation as well as TCR recognition, both important to determine the quality of the subsequent immune response. This allow us to understand the response towards influenza and HIV viruses, and more recently SARS-cov-2 virus. The molecular and biophysical features of the peptide antigens help us map the regions of the virus that are recognised by T cells, as well as determining the most stable and potent antigens that represent attractive target for therapeutics.

Using synthetic biology to generate hyper-stable vaccines

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Polypeptide vaccines effectively activate human T cells but suffer from poor biological stability, which confines both transport logistics and in vivo therapeutic efficacy. Synthetic biology has the potential to address these limitations through the generation of hyper-stable antigenic "mimics" that do not exist in the natural world. We have developed a platform based around non-natural chemistry and have used this platform to reverse engineer fully artificial T cell agonists that are that up to 6-fold more immunogenic than natural counterparts, including blueprints from influenza A, EBV, CMV and SARS-CoV-2. These non-natural vaccines are highly stable in human serum and gastric acid. In vivo, vaccinated mice were protected from lethal challenge. Moreover, the
synthetic agonists were immunogenic after oral administration without adjuvant. These proof-of-concept studies highlight the power of synthetic biology to expand the horizons of vaccine design and delivery.

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Effective targets of cross-species protective immunity against malaria identified by proteome-wide screening

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Vaccines against many diseases caused by complex pathogens are still not available despite intense research. The genome, proteome and transcriptome of a number of these challenging pathogens have been now elucidated, and provide the foundation for systematic genome-based approaches to identify target antigens for rational vaccine design. Using malaria as a model complex pathogen, we have developed and applied a strategy using T cell epitope prediction algorithms in reverse to identify and prioritize from the complete Plasmodium falciparum parasite proteome the subset of key antigens targeted by T cell responses from individuals with clinical immunity to malaria. We have also pursued proteome-wide screening using protein microarrays and sera from malaria-immune individuals to identify antigens targeted by antibody responses. Integrating our proteome-wide datasets showed that antigens preferentially recognized by T cells are distinct from antibody targets, suggesting that different vaccine approaches and antigen targets are required depending on whether antibodies or T cells are the desired vaccination outcome. Specific genomic, structural or physiochemical attributes could distinguish T cell versus antibody targets, facilitating the development of a predictive algorithm for immune class. Fourteen of the most highly ranked P. falciparum T cell antigens were evaluated for immunogenicity and capacity to protect against stringent cross-species P. yoelli parasite challenge in mice. Three immunization regimens were explored: homologous DNA-DNA, heterologous DNA-Adenovirus, and an innovative prime-target regimen designed to induce sustained T cell responses in the liver. Many of our novel P. falciparum antigens were effective targets of cross-species protective immunity, as evidenced by sterile infection-blocking immunity, reduction in liver-stage and blood-stage parasite burden or delay to onset of parasitemia. We have down-selected for clinical development a subset of antigens with maximum likelihood of inducing strain-transcending and cross-species protective immunity against malaria in humans. Such a rationally-designed genome-based vaccine would be expected to protect against all strains and all species of malaria.

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Rapid inactivation and deletion of adoptively transferred CTL upon encounter with a high number of antigen presenting lymphoma cells

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Adoptive T cell therapy (ACT) is a promising immunotherapeutic approach to fight cancer by transferring cytotoxic T lymphocytes (CTL), which specifically target and eradicate tumour cells. However, one major limitation of this therapy is the ability of tumours to interfere with the CTL through immune escape mechanisms, which may lead to poor CTL persistence and effector function. In a mouse model of B-cell lymphoma and ACT, we investigate the mechanisms underlying this failure. We found that tumour-antigen-specific CTL, upon transfer into a mouse with low tumour burden, successfully eradicate lymphoma cells, while upon encounter with a large tumour burden most of the CTL fail to survive and those that survive lose their effector functions. CTL death and loss of effector function are not induced by long-term persistence of antigen but were observed as early as 24-48 h after the adoptive transfer. Furthermore, CTL survival as well as killing ability and cytokine production were found to be dependent on the number of antigen expressing tumour cells rather than the total tumour burden. Our results describe a rapid deletion and inactivation mechanism of adoptively transferred CTL induced by encounter with a high number of antigen-presenting target cells.

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Stem cell memory like CAR-T cells persist better in vivo and induce solid tumour complete regression in combination with anti-PD1.

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CAR-T cells targeting solid cancers have had limited success, reasons for this include poor long-term persistence in vivo. To address this issue, we used naïve T cells to generate second-generation CAR-T cells recognizing the tumor antigen Lewis Y (LeY), termed 'early' CAR-T cells. To do this, purified naïve T cells were activated by CD3/CD28 soluble tetrameric antibody complex, retrovirally transduced (LeY scFv-CD3ζ-CD28 CAR) and expanded in IL-7/IL-15. The early-CAR-T cells comprised stem cell memory-like (CD95+, CD62L+, CD45RA+) and central memory phenotype (CD95+, CD62L+, CD45RA-) T cells with increased expression of ICOS, Ki67, TCF1 and CD27. The early LeY CAR-T cell function was tested in vitro for cytotoxicity (Cr-release and degranulation), proliferation, and cytokine secretion by CBA, either de novo or following chronic stimulation for 1 month. The early-CAR-T cells showed potent antigen-specific cytotoxicity, and secreted significantly higher levels of cytokines
(IFN-γ, TNF-α and IL-2) and increased proliferation compared to conventional CAR-T cells. Importantly, after long-term chronic stimulation, early-CAR-T cells had significantly higher proliferative capacity compared to conventional CAR-T cells, and CD4+ CAR-T cells were critical for effective early CD8+ CAR-T cell proliferation capacity in vitro. Early CAR-T cells had significantly better in vivo tumour control (NSG-OVCAR3) compared to conventional CAR-T cells, this was associated with increased persistence of circulating CAR-T cells. Finally, early LeY-CAR-T cells combined with anti-PD-1 therapy completely regressed OVCAR3 tumours in the NSG mice. This was associated with a significantly increased percentage of circulating stem-cell memory like CAR-T cells in vivo.

In conclusion, our early CAR-T cells have better in vitro function and potent anti-tumor efficacy in vivo. Importantly, early-LeY-CAR-T cells combined with anti-PD1-treatment completely cleared LeY+ solid tumors in vivo. Finally, our early CAR-T cell production protocol is directly translatable for improving CAR-T cell efficacy in clinical trials for patients with solid tumours.

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**Hdac3 is an epigenetic inhibitor of the cytotoxicity program in CD8 T cells**

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Cytotoxic T cells play a key role in adaptive immunity by killing infected or cancerous cells. While the transcriptional control of CD8 T cell differentiation and effector function following T cell activation has been extensively studied, little is known about epigenetic regulation of these processes. Here we show that the histone deacetylase HDAC3 inhibits CD8+ T cell cytotoxicity early during activation and is required for persistence of activated CD8 T cells following resolution of an acute infection. Mechanistically, HDAC3 inhibits gene programs associated with cytotoxicity and effector differentiation of CD8 T cells including genes encoding essential cytotoxicity proteins and key transcription factors. These data identify HDAC3 as an epigenetic regulator of the CD8 T cell cytotoxic effector program.

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**TCR repertoire and transcriptome differs between optimal HLA-A*02:01- and high-risk HLA-A*24:02-restricted CD8+ T cell immunity against influenza A virus**

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Influenza viruses circulate annually and cause significant morbidity and mortality during seasonal epidemics. CD8+ T cells provide broad protective immunity against influenza viruses. The quality of CD8+ T cell response against viral infections and its protective capacity can be influenced by Major histocompatibility complex (MHC) class I polymorphisms, binding affinity of T cell receptor (TCR)/peptide-MHC-I complex, functional avidity, and the nature of the TCRβ repertoire. Here, we assessed the quality of CD8 T cells following activation and is required for persistence of activated CD8 T cells following resolution of an acute infection. Mechanistically, HDAC3 inhibits gene programs associated with cytotoxicity and effector differentiation of CD8 T cells including genes encoding essential cytotoxicity proteins and key transcription factors. These data identify HDAC3 as an epigenetic regulator of the CD8 T cell cytotoxic effector program.

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**The discovery of molecular regulatory mechanism of MR1 antigen presentation**

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Major histocompatibility complex class I-related protein 1 molecule (MR1) presents bacteria-derived vitamin B metabolites that are synthesised by wide range of microbes. Antigen presentation of MR1 is critical for the establishment, development and activation of highly abundant innate-like T cells, mucosal associated invariant T (MAIT) cells. Activated MAIT cells secrete inflammatory cytokines and acquire cytotoxic activity to clear the infection. Recent study also showed that MR1 presents tumour associated antigen (TAA) that expressed by wide range of tumour cells and promotes tumour killing. Hence, understanding of the regulatory machinery MR1 antigen presentation is important for the development of potential therapy for bacterial infection as well as cancer.

MR1 is maintained intracellularly as an endoplasmic reticulum (ER)-resident pool in the absence of infection, but encounter of metabolic ligands induces the trafficking of MR1 to the cell surface. MR1-ligand complexes stay on the cell surface for several hours after which they are internalised and mostly degraded. Elimination of surface MR1 is a requirement to terminate MAIT cell responses; so the cellular machinery that controls the internalisation of MR1 plays a critical role in the regulation of the MR1-MAIT cell axis.

With genome-wide CRISPR-Cas9 library screen, we have identified adaptor protein complex 2 alpha subunit (AP2A1) as the regulator of MR1 internalisation. Interaction of AP2A1 and MR1 was observed via proximity ligation assay. When AP2A1 is depleted in the cells, MR1 is internalised at much lower rate and presents antigen for prolong time. MR1 cytoplasmic tail consists of tyrosine-based motif which is highly conserved across mammalian species. However, this tyrosine-based motif is not a canonical sorting motif. In conclusion, MR1 internalisation is regulated by AP2 using a novel recognition motif. These results open the possibility of manipulating the internalisation of MR1 without affecting the trafficking properties of most AP2-regulated membrane proteins.

Optimising novel adjuvant formulations to promote protective immunity without reactogenicity

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Tuberculosis (TB) is the leading cause of disease from a single infectious agent, causing an estimated 1.5 million deaths and infecting an estimated 10 million individuals in 2018 (1). The current vaccine, Mycobacterium bovis Bacillus Calmette–Guérin (BCG), is particularly effective at reducing the incidence of childhood TB, miliary TB and meningitis (2) but overall BCG efficacy in the lungs varies greatly depending on age of administration, localisation of TB infection, geographical area where vaccine is administered, previous exposure to various mycobacteria and current immune status (3). To improve protective efficacy, subunit vaccine strategies are highly attractive avenues of inquiry, and thus the development of safe and effective adjuvants is a critical goal of TB vaccine development programs. In this study, we defined the immunostimulatory profile and protective effect against aerosol Mycobacterium tuberculosis infection of vaccine formulations incorporating the semi-crystalline adjuvant d-inulin (Advax) and CpG oligonucleotide and the QS-21 saponin (AdvaxCpQS) was the most effective combination, demonstrated by the capacity of CysVac2/AdvaxCpQS to significantly reduce the bacterial burden in the lungs of M. tuberculosis-infected mice. CysVac2/AdvaxCpQS protection was associated with rapid influx of neutrophils, macrophages and monocytes to the site of vaccination and the induction of antigen-specific IFN-γ/IL-2/γIFNαγβαpolyfunctional CD4+ T cells in the lung. When compared to the highly potent adjuvant combination of monophosphoryl lipid A and dimethyldioctadecylammonium bromide (MPL/DDA), AdvaxCpQS imparted a similar level of protective efficacy yet without the profound stimulation of inflammatory cytokines and vaccination site ulceration observed with MPL/DDA. Addition of DDA to CysVac2/AdvaxCpQS further improved the protective effect of the vaccine, which correlated with increased polyfunctional CD4+ T cells in the lung but with no increase in vaccine reactogenicity. The data demonstrate that Advax formulations can decouple protective tuberculosis immunity from reactogenicity, making them ideal candidates for human application.

Intrapulmonary vaccination with delta-inulin adjuvant stimulates non-polarised chemotactic signaling and diverse cellular interaction

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There is an urgent need for novel vaccination strategies to combat respiratory pathogens. Mucosal vaccine delivery is an attractive option as it directly targets the site of infection; however, preclinical development has been hindered by a lack of suitable mucosal adjuvants and a limited understanding of their immune effects in the lung environment. Herein, we define the early immune events following the intrapulmonary delivery of a vaccine incorporating the adjuvant delta-inulin. Analysis of the early inflammatory response showed vaccine-induced innate cell recruitment to lungs and local lymph nodes (LN) was transient and non-polarised, correlating with an increase in pulmonary chemotactic factors. Use of fluorescently labelled adjuvant revealed widespread tissue dissemination of adjuvant particles, coupled with broad cellular uptake and transit to the lung draining LN by a range of innate immune cells. Mass cytometric analysis revealed extensive phenotypic changes in innate and adaptive cell subsets induced by vaccination; this included identification of unconventional lymphocytes such as γδ-T cells and MAIT cells that increased following vaccination and displayed an activated phenotype. This study details a comprehensive view of the immune response to intrapulmonary adjuvant administration and provide pre-clinical evidence to support delta-inulin as a suitable adjuvant for pulmonary vaccines.

Unraveling immunity towards seasonal influenza vaccine in haematopoietic stem cell transplant recipients

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Seasonal influenza infections cause significant morbidity and mortality, with ~500,000 deaths annually. Current vaccination regimens are the best method to combat annual influenza disease, although efficacy varies across years and can be low in high-risk groups. Haematopoietic stem cell transplant (HSCT) recipients are at high risk of severe influenza infection and show impaired immune responses towards inactivated influenza vaccine (IIV), especially within six months post-HSCT. We investigated humoral immunity to the trivalent IIV in a cohort of HSCT recipients (n=18) in comparison to healthy controls (n=14). IIV significantly increased hemagglutination inhibition (HAI) titres in HSCT recipients, similar to healthy controls. A systems approach revealed increased levels of IgG1 and IgG3 antibodies towards influenza-specific haemagglutinin (HA) head, but not to neuraminidase, nucleoprotein or HA stem. IIV also increased the frequency of total, IgG class-switched and activated-memory (CD27+CD27−) influenza-specific B-cells, determined by recombinant HA-probes and flow cytometry. For those HSCT recipients who did not respond to the first dose, the second IIV dose did not improve humoral responses, although some donors reached a seroprotective HAI titre (≥40). Strikingly, selected HSCT recipients had profoundly higher antibody responses towards the A/H3N2 vaccine strain compared to healthy controls, and even showed cross-reactivity to antigenically-drifted A/H3N2 strains. Such superior responses were associated with a greater time-interval after HSCT and multivariant analyses revealed the importance of pre-existing immune memory. Overall, our study demonstrated efficient but variable immune responses to IIV across HSCT recipients. The findings provide insights into influenza vaccination strategies targeted to immunocompromised high-risk groups and the efficacy of two IIV doses.

Development of a DNA vaccine that provides protection against blood stage malaria

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Publish consent withheld

Structure–Activity Relationship of Lipopeptide-anchored Liposomes Vaccine Candidates

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Polyethylenimine: an intranasal adjuvant for liposomal peptide-based subunit vaccine against group A Streptococcus

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Introduction: Group A Streptococcus is one of the major causes of morbidity and mortality in developing countries. In more recent years, many researchers have made efforts to create effective vaccines against GAS. Most of the vaccine studies has focused on the subunit vaccines based on M protein. However, the application of whole protein as a vaccine antigen can lead to autoimmune reactions. As a result of this, epitopes derived from M protein have been utilized as an effective and safer alternative vaccine. In our study, we have selected J8 peptide that is derived from GAS M-protein and P25 peptide as B cell and T cell epitopes, respectively. Nonetheless, these epitopes could not induce strong immune responses on their own. To overcome this drawback, these epitopes have been attached to surface membrane of liposome by a lipid moiety. This work aims to examine the influence of special arrangement of cholesterol-antigen conjugates in liposomal formulation on system immunogenicity.

Methods: Synthesis of the peptide vaccine candidates containing J8 and P25 peptide epitopes was carried out by Fmoc SPPS method and cholesterol was conjugated to pure antigens at RT. Anchoring of the lipopeptides to a liposome membrane surface was performed using a film hydration method. An in vivo study was determined to evaluate the effectiveness of vaccine constructs upon intranasal immunization in mice to induce humoral immunity.

Results: Cholesterol was conjugated to four multiple antigen peptides containing B cell epitope (J8) and the T helper peptide (P25) successfully according to ESI-MS and analytical RP-HPLC. The particle sizes of synthesized liposomes were the same while the charges of particles were different. Additionally, the highest antibody titres (IgG) displayed in mice vaccinated with L4 (Ac-J8-K(CH)-P25+ liposome). Similar but slightly weaker responses were detected when mice treated with other liposome formulations.

Conclusion: There was no significant difference between the immunogenicity of lipopeptide-anchored liposomes although the positions of epitopes and lipid moieties were different.

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References:
Delivery of a peptide-based vaccine against Group A Streptococcus with the help of cell penetrating peptides

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M-protein based vaccine induces immunogenicity and protection from Streptococcus pyogenes when delivered on a high-density microarray patch (HD-MAP)

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Structure-activity analysis of a self-adjuvivanting cyclic lipopeptide delivery system for group a streptococcus peptide antigens

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Group A Streptococcus (GAS) infection causes a wide range of diseases, from minor throat infections to serious life-threatening invasive infections such as necrotising fasciitis. GAS is also the principle etiologic agent of rheumatic fever and rheumatic heart disease, which are responsible for the largest proportion of the over 320,000 GAS related deaths worldwide per year. The ever-present global burden of GAS and the large number of cases, which manifest to rheumatic heart disease, highlight the need for a safe and effective vaccine.

Here, we have investigated a cyclic decapeptide carrier incorporating a conserved B cell peptide epitope derived from the conserved region of the GAS M protein, a universal T-helper epitope and a synthetic toll-like receptor 2 targeting lipid moiety (lipoamino acid) as a possible self-adjuvanting GAS vaccine. Vaccine candidates were synthesised using a variety of standard techniques, including solid phase peptide synthesis, head-to-tail cyclisation and Huisgen 1,3-dipolar cycloaddition chemistry. Compounds were purified by preparative reverse phase high performance liquid chromatography (RP-HPLC) and characterised by analytical RP-HPLC and electrospray ionisation mass spectrometry.

A structure-activity relationship analysis of the cyclic lipopeptide vaccine candidate showed successful induction of GAS-specific IgG titres when administered subcutaneously without an additional adjuvant, with all lipidated vaccine candidates inducing antibody titres significantly higher than the negative control. Interestingly, a physical mixture of the vaccine components (instead of a conjugated vaccine) showed the highest antibody titres of all vaccine groups. Further, vaccine-generated antibodies were shown to effectively opsonise multiple strains of clinically relevant GAS bacteria. This proof-of-concept study showed the capability for a self-adjuvanting cyclic delivery system to act as a vehicle for the delivery of GAS peptide antigens to treat GAS infection. Results from this study provide a vaccine delivery system capable of inducing high titres of opsonic antibodies capable of opsonising several clinically significant strains of GAS bacteria.

Development of peptide based subunit vaccine for Tuberculosis

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Despite being curable, tuberculosis (TB) affects 10 million people annually and remains in the top 10 causes of fatality worldwide in recent years [1]. Mycobacterium tuberculosis (MtB), the infectious agent, typically infects the lung but can spread through the body and affect other organs as well. A person becomes infected after inhaling bacilli droplets exhaled from active TB patients. The infected person can become ill immediately or carry the infection latent. Latent infection can be reactivated once the host immune system is weakened. Vaccination has been critical in saving lives and reduce the burden of many infectious diseases in the last century. The current Bacillus Calmette Guerin vaccine offers inconsistent protection against the most prevalent form of TB, pulmonary TB. Peptide based subunit vaccine is a promising approach to combat TB as it minimizes microbial components, still elicits the desired immune response and avoids pathogenic reversion which is possible in vaccines comprised of live attenuated pathogens [2]. To cover all of the pathogen subtypes, the chosen epitope must be highly conserved. T helper cells are crucial linker between innate and adaptive immunity and hence are vital in peptide based vaccine. Immune responses without the T-helper component are inconsistent in heterogenous population and memory responses are diminished. The early secreted antigenic target 6 kDa (ESAT-6) is encoded in the chromosomal locus of RD1, an essential determinant of mycobacterial virulence and is present in pathogenic MtB but absent in BCG. However, ESAT-6 has inherently low immunogenicity and would require a suitable adjuvant or delivery system to evoke sufficient immune response. As the currently available adjuvants are toxic with adverse reaction potentials, we aimed to incorporate ESAT-6 into novel self-adjuvanting polyhydrophobic amino acid or polymer based delivery systems for the development of a peptide based nanovaccine against TB.


Prophylactic vaccination protects mouse sperm quality after Chlamydia muridarum challenge

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Despite being the most common bacterial sexually transmitted infection worldwide, with 127 million new infections per year, a Chlamydia vaccine remains elusive. Vaccination remains the most highly recommended method for eliminating chlamydial transmission and subsequent pathology, the most severe of which is infertility. Research has predominantly focused on females even though a similar incidence of infection occurs in both sexes, resulting in potential vaccine candidates being trialled only in females. Male infections are often underappreciated as the effects on male fertility are still being defined. Some data suggests that Chlamydia causes sperm DNA fragmentation, which is an important clinical indicator of fertility. We developed a male mouse model of prophylactic chlamydial vaccination. Male C57BL/6 mice were vaccinated intra-nasally with major outer membrane protein combined with Iscomatrix adjuvant (MOMP/IMX) or with IMX only, then challenged via the nasal route with Chlamydia muridarum, or PBS. At 1-, 2-, and 3-months post challenge the IMX adjuvant-only group had poor sperm quality demonstrated by lowered motility and oocyte-binding, abnormal morphology, and increased DNA damage. However, the MOMP/IMX vaccination group had comparable sperm quality to the non-infected control mice. Vaccination protected against infection-induced impairment of sperm motility, morphology, oocyte-binding, and DNA damage. Testicular and epididymal chlamydial burden were also reduced by vaccination. We hypothesise that vaccine-mediated protection is induced by CD4+ T cell involvement in both lower reproductive tract and testes, possibly combined with local IgA production.

Bile acid-based delivery system for lipopeptide vaccine against Group A Streptococcus

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Introduction: Group A Streptococcus (GAS) infection causes a variety of diseases in human, ranging from a benign throat infection to life-threatening rheumatic fever (RF) and rheumatic heart disease (RHD). However, there is no vaccines currently available on the market. Due to the autoimmune response associated with M-protein (major virulent factor of GAS), modern peptide-based vaccines approach has been widely investigated to develop safe vaccine against GAS [1]. This peptide-based vaccine requires immunostimulant (adjuvant) and/or delivery system to protect the antigenic peptide from degradation and induce the desired immunity [2]. Adjuvants in the market are either too toxic for human use (experimental adjuvants) or they are limited to particular applications (commercial adjuvants) [3]. Thus, we designed vaccine candidates that utilise J8 B-cell epitope and PADRE T-helper epitopes that were anchored to liposome via cholae acid as an adjuvant-free vaccine candidate [4].
Methods: Cholic acid-conjugates were synthesized using Fmoc-SPPS and self-assembled to form nanoparticles in water. In addition, the conjugates were also incorporated into liposomes and extruded via a 100 nm membrane to form uniform unilamellar vesicles. The size, size distribution (PDI), surface charge, morphology, and stability vaccine candidates were characterized using DLS and TEM. Immunological evaluation of cholic acid-conjugate and cholic acid-liposome was performed in C57BL/6 mice using the prime-boost vaccination strategy. The vaccine candidates were delivered intranasally. Antibodies produced by the immunized mice were measured and tested for their ability to opsonize different strains of GAS clinical isolates.

Results: Cholic acid-conjugate was successfully synthesized with high purity (>95%) and yield. Cholic acid conjugated to peptide epitope were able to self-assemble into rod-like nanoparticles. The conjugate was also incorporated into liposome. Both cholic acid-conjugate and cholic acid-liposomes were able to induce high J8-specific IgG1 titer that able to opsonize different GAS strains upon intranasal immunisation. Cholic acid was able to enhance the immune response of targeted antigen and show stronger adjuvanting capacity than traditional self-adjuvanting lipid.

Conclusion/Implications: Cholic acid (a human bile acid) upon conjugation to GAS B-cell epitope self-assembled into rod-like nanoparticles, which induced opsonic antibody production in mice. Thus, we have shown for the first time that human-derived lipid, cholic acid, can act as a built-in immunoadjuvant for simple intranasal vaccination.


Whole blood immunophenotyping uncovers immature neutrophil-to-VD2 T-cell ratio as an early marker for severe COVID-19

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SARS-CoV-2 is the novel coronavirus responsible for the current COVID-19 pandemic. Severe complications are observed only in a small proportion of infected patients but the cellular mechanisms underlying this progression are still unknown. Comprehensive flow cytometry of whole blood samples from 54 COVID-19 patients reveals a dramatic increase in the number of immature neutrophils. This increase strongly correlates with disease severity and is associated with elevated IL-6 and IP-10 levels, two key players in the cytokine storm. The most pronounced decrease in cell counts is observed for CD8 T-cells and VD2 gd T-cells, which both exhibit increased differentiation and activation. ROC analysis reveals that the count ratio of immature neutrophils to VD2 (or CD8) T-cells predicts pneumonia onset (0.9071) as well as hypoxia onset (0.8908) with high sensitivity and specificity. It would thus be a useful prognostic marker for preventive patient management and improved healthcare resource management.

Development of C-type Lectin Receptor Targeting Subunit Vaccine Against Group A Streptococcus (GAS)

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Introduction: Antigen presenting cells (APCs) are the immune cells which process and present antigen to the T lymphocytes to mediate the immune response. Most common APCs are dendritic cell (DC) and macrophage which contain affluent C-type lectin receptors (CLRs) on their surfaces. The CLRs are the transmembrane receptors possessing the ability of binding of the sugar such as mannose containing ligands. Since subunit vaccines contain only the fragment of small antigen, these are instinctively less immunogenic. To overcome the poor immunogenicity, CLRs are the popular targets of subunit vaccines. Because if the carbohydrate moiety such as mannose is used in vaccine construct, it favours the uptake of antigen and consequently the following steps for improved immune response. GAS infection costs over 639,000 deaths annually mostly due to post-infection autoimmune disorder rheumatic heart disease (RHD). Over the decades research couldn’t result yet any GAS vaccine to resolve the GAS infection burden. Therefore, this study aimed to the development of CLR targeting subunit vaccine against GAS.

Experimental: Synthesis of peptide antigen and mannosylated ligands have been accomplished via Boc-SPPS (solid phase peptide synthesis). Peptides were purified by preparative HPLC and characterized by analytical HPLC and ESI-MS. Both antigen and mannose targeting ligands were anchored to the liposome delivery system to prepare nano vaccine. All formulations were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM).

Result: Peptide antigen and mannosylated targeting ligands were synthesized and purified (purity>95%). Peptide anchored liposome formulations were extruded to get the nanoparticle vaccine (<150nm, PDI <0.1).

Conclusion: Antigen and receptor targeting ligands have been successfully synthesized and used to prepare liposomal nanoparticle. In-vivo study is required to find out the most effective targeting ligand for antigen uptake by APCs.

References:

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Development of spherical, rod and worm shaped polymer based delivery system for peptide vaccine to treat Group A Streptococci (GAS) infection
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Group A Streptococcus (GAS), a gram-positive pathogenic bacterium has been accountable for variety of invasive and non-invasive diseases which may later lead to life threatening diseases like rheumatic fever and rheumatic heart diseases. Although research has been ongoing since 1923, vaccines against GAS are not yet available that requires cell-mediated and mucosal immunity stimulation. Thus investigation into new classes of vaccines has become one of the forefronts of medical research. Since traditional vaccines that use the whole GAS pathogen may trigger an autoimmune response, B-cell fragments from M-protein like J8 peptide epitope (QAEDKVKQREAKKQVEKALKQLEDKVQ) and universal T-helper epitope PADRE (AKFVAAWTLKAA) were synthesized to develop different peptide constructs.

One of the major challenges to overcome in the development of such peptide vaccines is the need for a strong immunostimulant (adjuvant) or delivery system to boost the vaccines’ immunogenicity as peptides poor immunogens on their own. Considering the associated toxicity of currently available adjuvants that are not only non-biodegradable but invariably invoke adverse reactions, allergic responses and inflammation, herein, our study focuses on a development of a potent polymer-based delivery system that is able to induce immune responses against the peptide epitope, without the use of any additional adjuvant.

The antigen PADRE-J8 was synthesized using solid phase peptide synthesis which was either physically mixed or chemically conjugated to hydrophobic polymers (poly(methacrylate) or poly[(N-isopropylacrylamide nanoparticles)]). All vaccine candidates formed nanoparticles and were capable of inducing the production of opsonic epitope-specific antibodies, following the subcutaneous immunization in mice. Among three different shapes of nanoparticles; sphere, rod and worms, it was found that the rod shaped nanoparticles specifically elicted a highest level of IgG titres than CFA based adjuvant after the first boost. Thus, we have successfully demonstrated that the shape plays vital role in inducing strong humoral immune responses.

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Human neutralising antibodies elicited by SARS-CoV-2 non-D614G variants offer cross-protection against the SARS-CoV-2 D614G variant
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Random mutations in the viral genome is a naturally occurring event that may lead to enhanced viral fitness and immunological resistance, while heavily impacting the validity of licensed therapeutics. A single point mutation from aspartic acid (D) to glycine (G) at position 614 of the SARS-CoV-2 spike (S) protein, termed D614G, has garnered global attention due to the observed increase in transmissibility and infection rate. Given that a majority of the developing antibody-mediated therapies and serological assays are based on the S antigen of the original Wuhan reference sequence, it is crucial to determine if humoral immunity acquired from the original SARS-CoV-2 isolate is able to induce cross-detection and cross-protection against the novel prevailing D614G variant. In this study, profiling of the anti-SARS-Cov-2 humoral immunity reveals similar neutralisation profiles against both S protein variants, albeit waning neutralising antibody capacity at the later phase of infection. These findings provide further insights towards the validity of current immune-based interventions.

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Development and evaluation of a peptide based liposomal blood-stage malaria vaccine
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**Plasmodium-induced interferon-γ restricts O’nyong-nyong virus replication and dissemination in mouse tissues**

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O’nyong-nyong virus (ONNV) is an alphavirus transmitted by main malaria vectors (Anopheles spp.) suggesting the possibility of co-infections in endemic areas. However, the potential pathological outcomes of such interactions in humans remain unknown. Using murine malaria models, we investigated the effects of pre-existing Plasmodium infections in the development of ONNV-associated pathologies. We report that Plasmodium infections suppressed hallmark ONNV-induced footpad swelling and viremia. In-vivo monitoring of ONNV replication kinetics in infected footpads revealed reduced viral load during the first 24 hours post-virus infection. Flow cytometry analysis showed that pre-existing Plasmodium infections rendered footpad CD45+ cells (monocytes/macrophages) and CD45+ cells (fibroblasts, myoblasts and endothelial cells) less susceptible to ONNV. Quantification of immune soluble factors in serum and footpad tissue lysates of Plasmodium-infected mice yielded increased levels of antiviral interferon gamma (IFNγ) which prompted further assessment of its role in the suppression of ONNV replication. Antibody blockade of Plasmodium-induced interferon-γ or co-infection in IFNγ-deficient animals restored ONNV replication to similar levels than animals singly-infected by ONNV. These results suggest that Plasmodium-induced interferon-γ restricts ONNV replication in mice.

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**Sensitive detection of total anti-Spike antibodies and isotype switching in asymptomatic and symptomatic COVID-19 patients**

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Early detection of infections is crucial to limit the spread of coronavirus 2019 disease (COVID-19). Here, we developed a flow cytometry-based assay to detect SARS-CoV-2 Spike protein (S protein) antibodies in COVID-19 patients. The assay detected specific IgM and IgG in COVID-19 patients and also the acquisition of all IgG subclasses, with IgG1 being the most dominant. The antibody response was significantly higher at a later stage of the infection. Furthermore, asymptomatic COVID-19 patients also developed specific IgM and IgG, with IgG1 as the most dominant subclass. Although the antibody levels were lower in asymptomatic infections, the assay was highly sensitive and detected 97% of asymptomatic infections. These findings demonstrated that the assay could be used for serological analysis of symptomatic patients, and also as a sensitive tool to detect asymptomatic infections, which may go undetected.
Development of glycan-based vaccine candidates to protect against *Streptococcus pyogenes*

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Introduction:

1. *pyogenes* is a widespread primary infective agent in humans and causes ~700 million human infections each year, beginning from mild streptococcal pharyngitis (strep throat) to invasive streptococcal pneumonia, necrotizing fasciitis, toxic shock syndrome, and myositis [1]. In addition to causing post-streptococcal sequelae, such as rheumatic heart disease (RHD), rheumatic fever (RF) and acute glomerulonephritis. The estimated total economic burden of GAS-induced disease in Australia, according to 2015 birth rates, was more than 44 million AUD with GAS-induced heart diseases, RF and RHD contributed more than 16 million AUD [2, 3]. Carbohydrate-based vaccines have been proven to be the most promising subunit vaccine candidates, as the bacterial glycan pattern are different from that of the mammalian cells and show more conservancy amongst pathogen serotypes than that of the protein components. In this work, we implemented the outcomes of the recent contributions of reverse vaccinology to develop a glycan-based subunit vaccine against *S. pyogenes*. We adapted a facile method for the synthesis of the glycotopes of *S. pyogenes* to be later conjugated to self-adjuvanting lipo-peptide and cyclic peptides.

Methods:
We have adapted a robust method to prepare the glyotope of *S. pyogenes* which is a blend of liquid-phase and solid-phase glycan synthesis requiring a single orthogonally-protected rhamnosyl monomer (Figure 1).

Figure 1: Solid phase glycan synthesis and automated glycan assembly

Results:
Orthogonally protected rhamnosyl monomer building blocks have been successfully prepared and the structure have been confirmed by NMR and mass spectrometry analysis. The scheme for glycopeptide synthesis via solid-phase glycan synthesis will also be presented.

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Linear B-cell epitopes in the spike and nucleocapsid proteins as markers of SARS-CoV-2 exposure and disease severity

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Background
Given the unceasing worldwide surge in COVID-19 cases, there is an imperative need to develop highly specific and sensitive serology assays to define exposure to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Methods
Pooled plasma samples from PCR positive COVID-19 patients were used to identify linear B-cell epitopes from a SARS-CoV-2 peptide library of spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins by peptide-based ELISA. Hit epitopes were further validated with 79 COVID-19 patients with different disease severity status, 13 seasonal human CoV, 20 recovered SARS patients and 22 healthy donors.

Findings
Four immunodominant epitopes, S14P5, S20P2, S21P2 and N4P5, were identified on the S and N viral proteins. IgG responses to all identified epitopes displayed a strong detection profile, with N4P5 achieving the highest level of specificity (100%) and sensitivity (>96%) against SARS-CoV-2. Furthermore, the magnitude of IgG responses to S14P5, S21P2 and N4P5 were strongly associated with disease severity.

Interpretation
IgG responses to the peptide epitopes can serve as useful indicators for the degree of immunopathology in COVID-19 patients, and function as highly specific and sensitive sero-immunosurveillance tools for recent or past SARS-CoV-2 infections. The flexibility of these epitopes to be used alone or in combination will allow for the development of improved point-of-care-tests (POCTs).

Lung endothelial cell antigen cross-presentation to CD8+ T cells: With an angle to hamper CD8+ T cells pathogenesis in malaria-associated lung injury
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Malaria-associated acute lung injury (MA-ALI) is a clinical complication of severe malaria. MA-ALI is characterized by edematous lung, along with marked infiltration of inflammatory cells and damaged alveolar-capillary barrier. However in MA-ALI, the pathogenetic mechanisms remain largely unclear. Murine models mimicking MA-ALI in humans are powerful tools to gain insights into the pathogenesis. Here, we show that C57BL/6 mice infected with Plasmodium berghei ANKA (PbA), a murine MA-ALI model, had increased transmigration of total and antigen-specific CD8+ T cells in the lung. Notably, in vivo antibody depletion of CD8+ T cells prevented lung injury. When we transferred antigen-specific CD8+ T cells to PbA-infected TCRβ-/- mice (devoid of functional T cells population), lung vascular leakage was recapitulated. Lastly, we further demonstrated that accumulation of parasitized erythrocytes led to lung endothelium cross-presentation of parasite antigen to antigen-specific CD8+ T cells, a process dependent on IFNg. In summary, lung vascular injury in MA-ALI is a consequence of the chronological occurrence of parasitized erythrocytes in the lung microvasculature, followed by antigen capture and processing by the activated endothelium, which cross-presents to antigen-specific CD8+ T cells infiltrated in the lung during PbA infection.