Research For Life

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Research Review 2019

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Editorial by Associate Professor Rebecca Grainger

Gender has been a hot topic in 2019. Gender equality, gender pay equity, gender identity, and more. Unfortunately, in many parts of the world, women face disadvantage and in many cases direct harm, due their gender. This has been recongised by the United Nation's Sustainable Development goal 5 of "Gender Equality". There are nine targets for action. It's sad to read that two are about preventing actual physical harm to women but positive that the other seven are about achieving equality for women in all aspects of domestic, community, economic and government life or achieving equitable access to health care. Fortunately, in New Zealand, women are faring better than some of their international sisters.

New Zealand has a strong and proud history of recognising the role of women in society. Just over a year ago, New Zealand celebrated "Suffrage 125". On the 19th of September 1893 New Zealand become the first self-governing country in the world to grant women the right to vote. Currently, women out-perform men in education; the gender pay gap for full-time employment is one of the lowest in the world (although we could still do better than the current 9.3%); and, we have a good track record for human rights and a comprehensive legislative framework that should protect women in New Zealand from discrimination. Given this background, its not suprising that women are leading our country.

Jacinda Ardern is New Zealand's third female prime minister and she has been in the spotlight internationally for her courageous and compassionate leadership. Many New Zealanders are aware we have a female governor general and female Chief justice, but not so many might know that the Prime Minister's Chief Science Advisor is a woman. Professor Juliet Gerrard took on this role in mid 2018. Professor Gerrard is a professor of biochemistry at the University of Auckland, where she still runs a lab. The primary function of the role of Chief Science Advisor is to give the Prime Minister strategic and operational advice on science and science

policy. Other tasks include increasing public awareness and engagement with science, promoting international relations via science, and alerting New Zealand to opportunities and threats of new scientific discoveries. Current work undertaken by the Chief Science Advisor's office includes the impact of plastics and microplastics, antimicrobial resistance, the use of 1080, impacts of climate change, 5G and health, genetic editing, and the effects of cannabis on health. It's interesting to note how much of this work relates to human health which, for each of us and for our communities, is our greatest asset. Not surprisingly then, how to best maintain and improve the health of New Zealanders is the focus of much of the research undertaken by New Zealand universities, independent research institutes, and government facilities.

Research For Life (RFL) is proud to support this type of medical and health-related research in Wellington. Many people are under the mistaken belief that students and staff at universities and government institutions have funds available for research. Even internal institutional funding requires competitive grant processes, and internal funds are limited. Most researchers spend quite signficant chunks of time grant-writing to fund their research. This is where RFL comes in, to support Wellington researchers undertaking exciting, cutting-edge research to further our understanding of how to maintain and improve human health.

Twice a year, the Research Advisory Committee, made up of respected and experienced scientists and clinicians from Wellington, reviews research grant applications and recommends to the RFL Board which projects could be funded. Funding is then allocated by the Board, according to funds available. Analysis of funding allocated from 2017-2019, showed that across the 27 successful applications, the principle investigators were 17 women and 10 men. While outstanding science is undertaken by scientists, irrespective of gender, it is

pleasing to see that women are leading the excellent science being undertaken in Wellington.

RFL is very proud to support all our grant recipients. I hope you enjoy reading this research report and learning more about the work of our present, and future, science leaders.

Research Advisory Committee (2019)

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Reports of Research Funded by Grants

Malaghan Institute of Medical Research

Assessment of the impact of a novel CSF1R inhibitor on immune profiles in different murine tissues with multiparameter flow cytometry

L Ferrer-Font and I F Hermans

Key Findings

By using multiparameter flow cytometry we could profile the immune cell populations within different organs after mice were treated with SN38114, a novel CSF1R inhibitor. Indeed, by using different routes of administration, we observed that the effect of the compound can vary, reflecting different rates of absorption and bioavailability, and showing that the intra-gastric route has a broader effect in the organs analysed than intra-peritoneal administration.

Objectives

Use multiparameter flow cytometry with high dimensional analysis to investigate the immune cell profiles of different organs, including tumour tissue, when mice are treated with SN38114, a novel CSF1R inhibitor.

Assess the impact of dose, and the route of administration on the immune profile of the different tissues.

Methods

Multiparameter flow cytometry is a powerful analytical tool that enables rapid measurement of multiple phenotypic characteristics of individual cells as they flow past beams of laser light in a focused fluid stream. Using this tool, we have developed a 20-colour panel to be able to determine the effects in different immune populations when PLX3397 (a commercially available CSF1R inhibitor) or SN38114 (developed by our collaborators) are administered to mice bearing established tumours. Using the Cytobank platform, we employed the t-

stochastic neighbour embedding (t-SNE) algorithm to generate a two-dimensional map where the distance between cells corresponds to their marker profile similarity.[1]

Treatment with SN38114 or PLX3397 by the intra-gastric route started at day 6 post tumour injection (at which time tumour volume was around 36 mm^2). SN38114 was administered at 45 mg/Kg, and PLX3397 at 40 mg/Kg. Organs (draining lymph node (dLN), spleen, fat tissue and tumour) were collected at day 5 after the first treatment. Cells were stained with a 20-antibody colour panel and analysed by flow cytometry using Cytek™ Aurora 3 laser flow cytometer, and t-SNE analysis was performed. By using a different administration route, the effect of the compound can vary. We examined if SN38114 showed differences when administered intra-peritoneally or intra-gastrically.

Results

Multiparameter flow cytometry panel design and optimisation

After two iterations we successfully designed and optimised a 20-colour panel which allowed us to detect the main cell types that could play a role in the response to CSF1R inhibition. Additionally, different data analysis algorithms (tSNE, SPADE, CITRUS and FlowSOM [1–3]) were tested in order to optimise both our visualisation and understanding of the data. For these experiments we decided to further utilise tSNE analysis.[2]

Immune cell profiles within different organs when mice are treated with SN38114 and impact of route of administration on immune profile in the different tissues.

Macrophages are a major cellular component of cancer-related inflammation and can influence cancer growth depending on their activation status. Classically (M1) and alternatively (M2) polarised macrophages exhibit anti-tumoural and pro-tumoural functions, respectively.[3] By intra-peritoneal administration, the main differences found when comparing treated mice (either with PLX3397 or SN38114), were in the fat tissue. As seen in

figure 1, we found a decrease in M2 type macrophages that was more pronounced in SN38114 than in PLX3397 treatment.

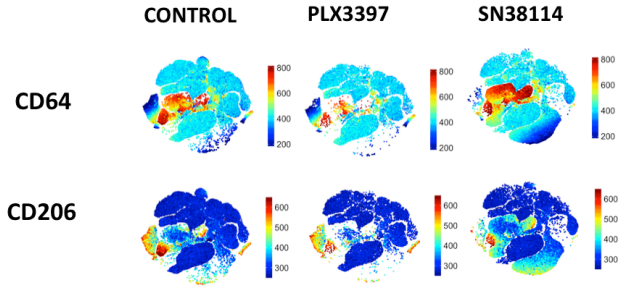

Figure 1. T-distributed stochastic neighbour embedding (vi-SNE/t-SNE) visualisation of a 20-color spectral flow cytometry panel from fat tissue cells; pregated on single, live, $CD45^+$ cells. The expression level for CD64 (macrophages) and CD206 (M2 type macrophages) markers is indicated on a colour scale in each tSNE plot (red indicates high level of expression). Mice were treated 5 consecutive days with PLX3397 (45 mg/kg, orally) or SN38114 (40 mg/kg, intraperitoneally).

By using a different administration route, the effect of the compound varied, reflecting different rates of absorption and bioavailability. We examined if SN38114 showed differences when administered intra-peritoneally or intra-gastrically. Intra-gastric route is the preferred route in the clinic due to ease of administration. In the case of fat tissue, we found a local effect when administering the compound intra-peritoneally with an increase of the M1/M2 ratio (reduction of M2 macrophages). While, when SN38114 is administered intra-gastrically, no changes were observed in the fat tissue (Figure 2A), but there was a reduction of M2-type macrophages in the dLN (Figure 2B) and spleen (spleen graphs not shown because they were very similar to the LN organ). Furthermore, no significant differences were found regarding tumour tissue.

Unfortunately, these results couldn't be reproduced because of the loss of the activity when using new batches of SN38114. At this stage, our chemist collaborators are analysing the purity and the activity of the compound. We are expected to understand

the batch to batch SN38114 differences soon, which will allow us to continue with the project.

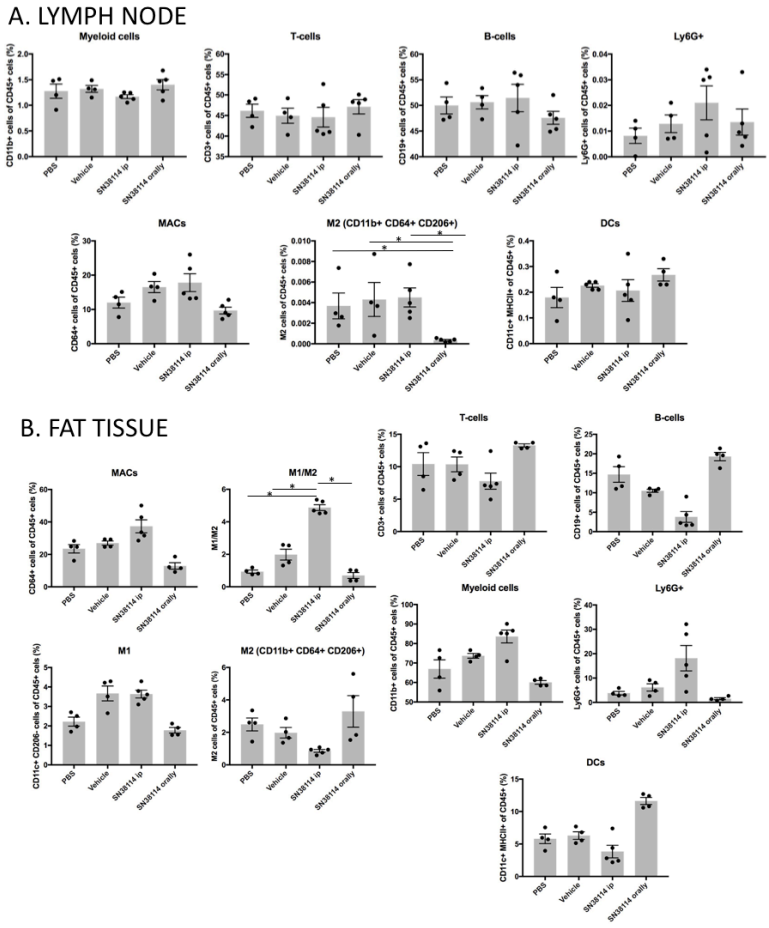

Figure 2. M1 and M2 macrophages phenotypes expression when comparing PLX3397 or SN38114 treated mice. SN38114 in 20% Hydroxypropyl-β-cyclodextrin (HP-β-CD) (40mg/Kg i.p.) and PLX3397 in 10% DMSO in PBS (45mg/Kg intra-gastrically) were administered every day during 5 consecutive days. At day 6 samples were collected and analysed by flow cytometry. n=5.

Conclusion

The panel design and optimisation have been successful, and we have found some significant differences when comparing organs

and routes of administration. However, despite encouraging results with the first batch of compound, batch to batch differences have made us put this project on hold while we wait for the chemist collaborators to understand the loss of activity of the new batches.

References

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Functionally defining human mucosal associated invariant T (MAIT) cell subsets ex vivo.

K Woods and O Gasser

Key findings

A large flow cytometry panel was used to generate information about MAIT cell number and function in 110 human blood samples. Faecal microbiome data was obtained from matched stool samples. These two datasets were combined to compare the composition of the microbiome with the function of MAIT cells.

Objectives

The objective of our study was to look in detail at a subset of human immune cells (called Mucosal-Associated Innate-like T cells (MAIT) cells), which have been described relatively recently (2003), and which are under-studied compared to other immune cell types. These cells are important, because they are enriched at the body's environmental barrier surfaces, such as the skin, and the lining of the lungs and the gut. We initially wanted to investigate how different sub-types of MAIT cells behaved,

however a recent publication has largely described this, causing us to develop our studies in a slightly different direction.[1] Because MAIT cells reside in the gut, and because they are activated by products broken down by the microbes in the gut (the microbiome), we set out to investigate how MAIT cells behaved in people with different microbiomes. We wanted to see if a certain microbial profile could be associated with a certain type of MAIT cell behaviour, because the knowledge of how MAIT cells are changed by the microbiome, and what kind of microbiome makes "good" MAIT cells, could potentially be used to develop therapies for a number of different diseases associated with the microbiome.

Method

We previously sequenced the faecal microbiome of 110 human donors.[2] Using matched blood samples from these patients, we looked at MAIT cell number and functional type, in order to compare this with the microbiome. The main method we have used to do this is by flow cytometry. This is a method where cells are "tagged" with antibodies attached to different fluorescent colours. When these cells are run through a flow cytometer, lasers in this machine enumerate the cells which the antibody has bound to. Because we know the markers that are on the surface of many different immune cells, we can use a pool of differently coloured antibodies to separate out these cells, count them, and determine their function. At the Malaghan Institute we are the only group in New Zealand to use a spectral flow cytometer which allows us to routinely combine 25-30 differently coloured antibodies (traditional flow cytometers allow 8-12). We have designed a pool of 24 differently coloured antibodies to enable us to look at 7 immune cell types in one human blood cell sample (Figure 1). Using this pool of antibodies, we can also look at other markers on each immune cell population which gives us more information about the function of each cell (*e.g.* are they activated? how are they acting when they are activated?).

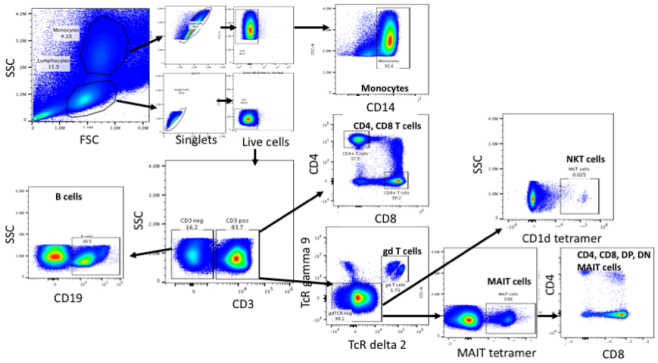

Figure 1 – A 24 colour flow panel allows gating of 7 major cell subsets. Human PBMC samples were incubated with a MAIT specific tetramer for 30 min at room temperature. Cells were washed and incubated with fluorescent antibodies for 20 min at 4oC. Cells were washed and analysed by flow cytometry. The subsequent gating strategy is shown.

Results

When we labelled human blood cells with antibodies and looked at the different immune cell populations, we found variability in the number of total MAIT cells across donors, from ~0.5% to over 10% of total T cells (Figure 1). These numbers are similar to previous reports for MAIT cell numbers in human blood. We have done some initial analyses comparing the number of total MAIT cells, as well as three previously described MAIT cells subsets, with two different microbial species. The percentage of *Bacteroides* (which has been previously described as MAIT cell activating) and *Prevotella,* in faecal samples was determined. We graphed the number of MAIT cells against the percentage of either bacterium in matched blood/stool samples (Figure 2). We found no correlation between the presence of these two bacteria, and MAIT cell numbers for total MAIT cells or any of the three subsets analysed (Figure 2 a-d, h-k). Next, we looked at the effect of these bacteria on MAIT cell activation. We used the marker CD69 to measure MAIT cell activation, and once again compared this with the percentage of either bacterium in matched stool samples. However, we again found no correlation between the presence of either bacterium and activation of any subset of MAIT cells (Figure 2, e-g, l-n).

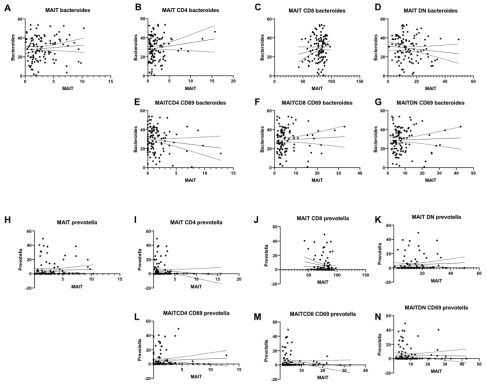

Figure 2 – Association of MAIT cell number and activation status, with presence of two bacterial species in matched blood/stool samples. Total MAIT cell numbers (a, h), as well as three distinct subsets (b-c,i-j) in human blood samples were determined by flow cytometry. The percentage of two different microbial species; *Bacteriodes* (a-g) and *Prevotella* (h-n) as components of the microbiome in matched stool samples were determined, and compared with MAIT cell numbers (a-c, h-j). MAIT cell activation (expression of CD69) was also determined by flow cytometry, and compared with bacterial load for these two bacteria (e-g, l-n).

Since we have labelled cells with a large marker panel with this antibody pool, we used a high-dimensional data analysis software tool to allow us to more clearly visualise our data, and to discriminate differences between cells across several markers. This software tool is call tSNE. It works by clustering groups of cells with similar properties together, and thus allows us to compare the representation of cells with different properties within our sample in one plot. In this way, it is possible to draw out associations between MAIT cells, and their functional markers, which we may not otherwise distinguish. An example is shown in Figure 3. Here, we have run tSNE, which resulted in the cluster pattern shown. Next, we used a “heatmap” function, where we looked at each antibody individually, and the cells most strongly expressing that marker were shown along a colour scale from red (strongest expression) to orange, yellow, green, and blue (weakest expression). In this way, we could identify which cluster represented different immune cell populations and draw gates around them to mark their positions (Figure 3, middle row). Finally, we used the heatmap function to identify which cells were expressing the other (functional and activation) markers in our panel (Figure 3, bottom row). In this way we can show, for example, that about half of the MAIT cells express the marker CD56, whereas none express CD103, which is found only in a small sub-cluster of CD8 T cells (Figure 3, bottom row).

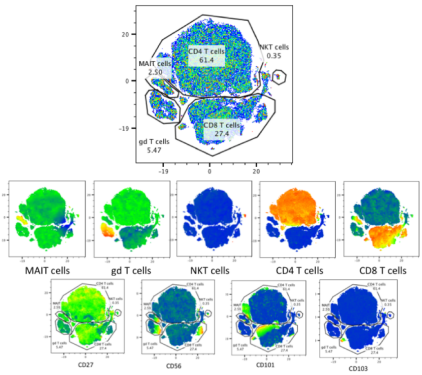

Figure 3 – High dimensional data analysis distinguishes cell subsets and activation markers across donors. CD3 positive cells were gated for all samples (as in Figure 1), and files were concatenated. tSNE was used to cluster groups of similar cells together (Cytobank software), and a heat map function (FlowJo software) was used to gate which clusters represent which cell types (top panels). Subsequent analyses for the expression of activation markers allows visualization of which cell types are expressing which activation markers (bottom panels).

Since one of our major research questions is to address the interplay between the makeup of the microbiome and MAIT cells, we next compared these two factors by tSNE. We combined the flow cytometry data, this time just looking at just the MAIT cells (red box, Figure 1) and the microbiome data, *i.e.* the percentage of each bacteria making up the faecal microbiome in each donor. We compared the expression of different MAIT cell markers with each bacterial species we found in the microbiome. We currently have data for MAIT cell marker expression in the presence of 16 different bacterial species, and three are shown in figure 4 as examples. Here, expression of each MAIT cell marker is indicated by the gates which have been drawn in, and expression of each bacteria by the heatmap function. Using this data, we can see, for example, that presence of more *Bacteroides* may be correlated with increased expression of GATA and IL-4 by MAIT cells (Figure 4, top panels). The reverse situation is seen for *Faecalibacterium,* where fewer of these bacteria are associated with GATA and IL-4 expressing MAIT cells (Figure 4, bottom panels).

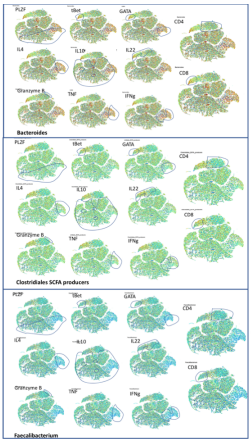

Figure 4 – High dimensional data analysis allows association of MAIT cell phenotypes with microbiome bacterial composition across patients. Human PBMC samples were gated specifically on MAIT cells. Microbiota information was added to each patient sample and files were concatenated. tSNE was used to cluster similar groups of cells together (Cytobank software), and a heat map function (FlowJo software) was used to gate which clusters represent which MAIT cell phentoype. Subsequent analyses for the presence of bacteria types allow visualization of MAIT cell phenotype correlations with bacterial types (shown here examples for 3 different bacteria). SFCA=short chain fatty acids.

Conclusion

For this research project, we have completed all data collection. This included the optimisation of a large flow cytometry panel, and its use to stain and analyse blood samples from 110 donors. We have also obtained faecal microbiota data for all of these patients. Having obtained all of this data, we have commenced comparative analyses between MAIT cell function and the composition of the microbiome. Due to the large-scale data sets we have obtained in the course of this project, these analyses are ongoing. Nevertheless, it is clear to us that meaningful associations may be made. For example, GATA is transcription factor which controls the expression of cytokines, such as IL4, which are associated with a certain type of immune response – a "type 2" immune response. Because of this, we would expect these two markers to behave in a similar manner in terms of when/where they are expressed. In our data we found that both of these markers were associated with high levels of Bacteroides and with low levels of *Faecalibacterium*. We are currently collaborating with bioinformaticians to ensure we maximise our analyses of this data and anticipate a paper will be formulated later this year to report the complete results of this study.

In addition to our MAIT cell analyses, the data we have already collected will also allow us to compare the number and function

of other immune cell subsets with the composition of the microbiome. In particular, gamma delta T (gd T) cells, and natural killer T (NKT) cells (Figure 1) are two cell types described as having associations with the microbiome. We have not yet looked at this in our dataset but anticipate that this will generate additional novel information.

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Victoria University of Wellington

The response of immune cells from patients with progressive multiple sclerosis to the drug MIS416, and the impact of genetic changes in the NFκB pathway

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Key Findings

We observed that immune cells from patients with MS had significantly altered activity in terms of their cellular signalling. Two key transcription factors (TF) that facilitate signalling in immune cells (STAT1 and NFκB) were much lower in MS participants, and our drug further changed their balance and the number of cytokines produced by these cells.

We also observed that when we classified our participants based on whether they had a specific mutation in the gene NFKBIA, that an impact on immune factors from the presence of the mutation could be seen, and that the mutation was affecting the response of immune cells in terms of cytokine production and TF activation.

Objectives

Our objective in conducting this research was to contribute to the understating of the effects of a drug (MIS416) on the immune system by describing its impact on peripheral blood responder cells named monocytes. Furthermore, we aimed to obtain data on a range of other immune cells at the same time, using a new spectral cytometry platform to facilitate this work. We also aimed to establish if a mutation in NFKBIA, which is a regulating gene that encodes a protein inhibitor of NFκB pathway, could impact the way cells respond to immune signals.

Methods

We obtained a blood sample from 27 people with progressive

MS, and from 9 healthy controls. We then performed cell culture experiments on their blood cells to profile the responses from these cells to a range of experimental conditions, including treating the cells with the drug MIS416. Data on the cells was acquired using the flow cytometry technique, which is a well-established method that allows for the analysis of a range of immune factors in cells. We used a newly released platform for this analysis, the Cytek Aurora, which provided significantly improved resolution of the responses in cells, such that we could observe the responses of many different types of cell at the same time.

We also extracted DNA from the cells of each patient and sent the material for Sanger sequencing of the NFKBIA gene. We then used that data to assign participants to groups based on this genetic sequence. This allowed us to compare the immune data between the genetic groups and allowed us to establish the impact of the genetic mutations on the immune responses seen in the participants' cells.

Results

The two classes of immune molecule we observed were TF and cytokines. TF are signalling proteins that communicate the external signals sensed by the cell to the genetic machinery that ultimately produces the cells' responses to such external stimuli. When TF are activated, they instigate the expression of a range of genes that control the immune response. We observed that MS patients had overall less activity of some TF, specifically NFκB and STAT1, and that MIS416 could further change the level of activity in some participants. MIS416 was able to reduce the activity of STAT1 and STAT3 in treated cells.

As for cytokines (which are those key molecules that mediate communication between immune cells and with other tissues), we observed that MIS416 had a dramatic impact on the amount produced. We observed that untreated MS patients had increased levels of TNFa, a potent immune activator, much more so than healthy participants. We also showed that MIS416

proved to be particularly effective at increasing production of IL-10, a known immune dampening signal.

When we compared groups of participants based on their NFKBIA mutation status, we observed the impact of the mutation on immune factors. As anticipated, untreated cells from patients with the mutation had increased activity of the TF pathways and this appeared to have follow-on repercussions on cytokine expression also. MIS416, which has a method of action that is centred on NFκB activation, also effected patients with mutations differently to those who had no mutation.

Conclusion

We conclude that the effects of MIS416 on the immune system may result in a differential response to treatment in individuals with progressive MS due to the impact of NFκB pathway changes. The large increase in IL-10 from MIS416 could offer a way to reduce runaway immune activity classically observed in MS but, as not all patients are likely to respond to the drug, a personalised treatment regime would be required.

Characterisations of B cells in Acute Myocardial Infarction

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Key Findings

Our preliminary data suggests that changes in the distribution of the transitional, naïve and memory B cell populations occur in heart attack patients when compared to healthy subjects. Our current observations are consistent with pre-clinical literature suggesting that B cell subpopulations are dynamically changing in the early inflammatory response in heart attacks.

Objective

An acute myocardial infarction (AMI, also known as a heart attack), is the acute clinical presentation of coronary artery disease (CAD), where an obstruction of the coronary arteries that surround the heart limits blood supply to the heart. Both the progression of CAD and the extent of myocardial damage during AMI is strongly driven by activation of the immune system and the inflammatory response.

The contribution of the adaptive immune system to the inflammatory response following a heart attack is not well understood (Sattler, Fairchild *et al.* 2017). Recent reviews suggest that adaptive immune cells could play a detrimental role by dysregulating the immune response following a heart attack (Flego, Liuzzo *et al.* 2016, Sattler, Fairchild *et al.* 2017). The involvement of B cells (an adaptive immune cell) in this response has yet to be established. Animal models suggest that B cell numbers increase following AMI and that depletion or addition of B cells can shape the inflammatory response and subsequent damage to the heart tissue (Goodchild, Robinson *et al.* 2009, Yan, Anzai *et al.* 2013, Zouggari, Ait-Oufella *et al.* 2013). However, there remains a lack of clinical studies investigating the role of this cell type following a heart attack.

To examine the role of B cells in humans, it is first necessary to determine how the number of B cell subpopulations change in patients with CAD and with AMI, compared to age- and sex-matched healthy subjects. In this study we have developed a method to identify circulating peripheral B cells in patient groups across the CAD pathology (AMI vs. CAD vs. healthy).

We aim to determine whether the distribution of each of peripheral B cell subsets differs in acute myocardial infarction (AMI) subjects when compared to age- and sex-matched stable coronary artery disease (CAD) subjects and healthy subjects.

Methods

We are currently actively recruiting participants into this study.

We have completed recruitment of AMI patients, with 20 subjects enrolled. A further 20 patients with stable CAD and 20 healthy subjects that are matched for age (± 5 years) and sex, are being actively recruited. We have currently recruited 9 CAD patients and 12 healthy subjects. Current demographics of recruited participants are in Table 1.

Table 1. Participants Demographics Table

Cohort:	Healthy Participants (n=12)	Stable CAD Patients (n=9)	Heart Attack Patients (n=20)
Age:	54.4 (±6.6)	59 (±6.7)	59.75 (±11.4)
Sex:	M: 7 / F: 4	M: 6 / F: 3	M: 15 / F: 5
BMI:	n/a	31.95 (±4.6)	31.8 (±9.3)
Ethnicity			
European:	11	6	15
Maori	0	1	3
Pacific Island	1	0	0
Chinese	0	0	0
Indian	0	2	1
Other:	0	0	1
Medical History			
Family History:	n/a	2	5
Diabetes:	0	2	2
Hypertension:	n/a	7	9
Dyslipidaemia:	n/a	5	12
History of Angina:	n/a	6	2
Previous MI:	n/a	1	4
CABG:	n/a	0	3
Smoking Status			
Never Smoked:	10	5	9
Past Smoker:	1	3	6
Current Smoker:	1	1	5
Type of MI			
STEMI	n/a	n/a	3
NSTEMI	n/a	n/a	11
PAMI	n/a	n/a	6

For each study participant, a single blood sample is taken on the day of enrolment. Peripheral blood mononuclear cells (PBMCs), which include the B cell populations, are isolated from this blood sample and this sample is cryopreserved until time of analysis.

Analysis of B cell subpopulations is performed using flow cytometry. This technique uses antibodies that bind to cell surface markers on the individual cells, and these antibodies are linked to fluorescent probes that we can detect using flow cytometry. The PBMC samples are stained with a panel of

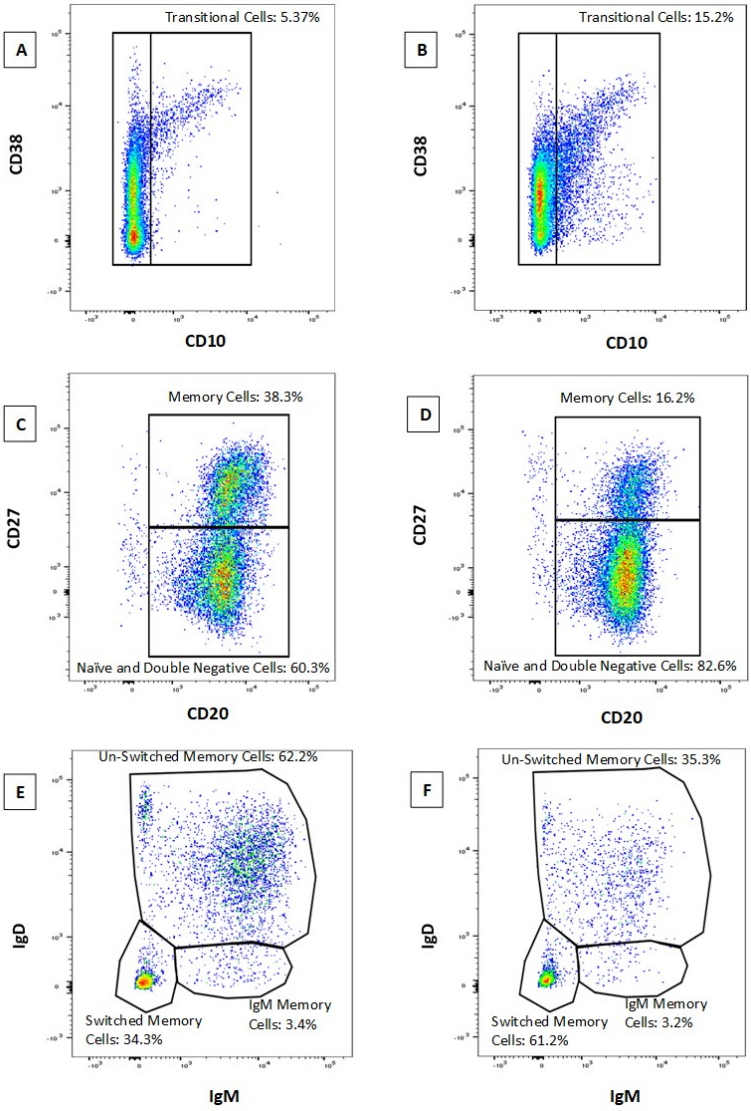
antibodies that will be used to identify seven B cell subpopulations. Each of these subpopulations is defined in Table 2. Optimisation of the methodology for this B cell characterisation has been completed, and at this stage we have analysed samples from four AMI subjects and 4 healthy controls.

Table 2. B Cell Subpopulation Surface Markers Used for Identification

Peripheral B Cell Subsets	Surface Markers
B Cell Populations	$CD3^{-}CD19^{+}$
Plasma Cells	$CD10^{-}CD38^{++}CD27^{++}CD20^{-/+}$
Naïve B Cells	$CD10^{-}$ $CD38^{+/-}CD27^{-}$ $CD20^{+}$ IgD^{+} $IgM^{+/-}$
Memory B Cells	$CD10^{-}$ $CD38^{+/-}CD27^{+}$ $CD20^{+}$
Memory B Cell - Unswitched	IgD^{+} IgM^{+}
Memory B Cell - Switched	IgD^{-} IgM^{-}
Memory B Cell - IgM Memory	IgD^{-} IgM^{+}
Double Negative B Cells	$CD10^{-}$ $CD38^{+/-}CD27^{-}$ $CD20^{+}$ IgD^{-} IgM^{-}
Transitional	$CD10^{+}$ $CD38^{+/++}CD27^{-}CD20^{+}$

Preliminary Results

We have conducted analysis of B cell subpopulations from four healthy (mean age: 53±4.8, Sex: F: 1) and four AMI (mean age: 56±6.7, Sex: F: 1) participants. Initial analysis reveals qualitative differences between frequencies of B Cell subpopulations in AMI and healthy subjects, as seen in the representative flow cytometry plots from two analysed samples (Figure 1).


A
Transitional Cells: 5.37%
CD38
CD10
B
Transitional Cells: 15.2%
CD38
CD10
C
Memory Cells: 38.3%
Naïve and Double Negative Cells: 60.3%
CD27
CD20
D
Memory Cells: 16.2%
Naïve and Double Negative Cells: 82.6%
CD27
CD20
E
Un-Switched Memory Cells: 62.2%
IgM Memory Cells: 3.4%
Switched Memory Cells: 34.3%
IgD
IgM
F
Un-Switched Memory Cells: 35.3%
IgM Memory Cells: 3.2%
Switched Memory Cells: 61.2%
IgD
IgM

The phenotype of B cells in AMI patients quantitatively differed from healthy controls in the following ways: the percentage of transitional B cells was higher in AMI patients (8.4±2.4%) than in healthy controls (7.5±2.4%), of mature B cells, a lower percentage were memory cells in AMI patients (18.8±7.5%) than in healthy subjects (27.0±6.1%). Conversely, the percentage of mature B cells that were naïve in the heart attack patients was higher (75.3±7.6%) than in the healthy subjects (65.2±6.2%). Of the memory cells, a higher proportion had switched in heart attack patients (61.4±2.8%) than in healthy subjects (42.2±10.7%).

Our quantitative evaluation will be completed once recruitment of all 60 participants (n=20 for healthy, CAD and AMI) is complete. We anticipate completion of recruitment by the end of September 2019.

Conclusion

We have optimised a methodology for characterising B cell subpopulations in AMI, CAD and healthy subjects. Our preliminary data suggests that changes in the distribution of the transitional, naïve and memory B cell populations occur in AMI patients when compared to healthy subjects. Once participant recruitment is complete, we will complete flow cytometric analysis for all 60 participants and examine changes in frequency of each B cell subpopulation statistically. Our current observations are consistent with pre-clinical literature suggesting that B cell subpopulations are dynamically changing in the early inflammatory response in AMI.

Aptabiotics - targeted nanomedicines for treating bacterial infections

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Introduction

P. aeruginosa is an opportunistic pathogen that was ranked second on the list of drug-resistant bacteria that pose the greatest risk to human health by the World Health Organisation in 2017. The rise in antibiotic resistance constitutes one of the greatest emerging threats to human health that requires urgent development of new drugs and treatment approaches. While the use of silver for medicinal purposes is ancient and well established in modern medicine, its use as an antimicrobial is limited by its therapeutic index. We reasoned that targeting of nanosilver to the pathogens using DNA aptamers could potentially increase the utility of silver as an antimicrobial agent. We report the use of a *P. aeruginosa* specific DNA aptamer to deliver a highly toxic dose of DNA chelated nanosilver as a targeted antimicrobial to bacteria grown planktonically and in biofilms.

Aim

To further develop and functionalize the DNA aptamers that we have developed to live PA, specifically, we aimed to:

1. Conjugate DNA sequences onto the aptamers that are able to act as scaffolds for forming silver nanocluster.
2. Optimize the placement and structure of the nanocluster scaffolding sequence to maximise antimicrobial activity
3. Evaluate aptamer-silver conjugates for the ability to kill PA, and disrupt biofilms.

Methods

Polycytosine rich sequences were conjugated either to the 5’ or 3’ end of the aptamers, either with or without a poly-T spacer for evaluation for antimicrobial activity against PA. Additionally, a split nanocluster forming sequence that spanned the stem

region of the aptamer was prepared for evaluation. Antimicrobial activity was monitored by following the increase in turbidity of cultures for 20hrs, using standardized criteria for determining MIC and MBC. Depolarisation of cells was determined by propidium iodide staining and flow cytometry. Biofilm disruption and killing of biofilm cells was determined by crystal violet staining of biofilms grown on Calgary pegs and plating to determining the number of colony forming units.

Results

The aptamer-nanosilver conjugates that we call 'aptabiotics' have potent antimicrobial activity as determined by bacterial growth curves and viability staining, with aptamer targeted silver being more effective at killing *P. aeruginosa* than the equivalent untargeted silver. The aptabiotics have an IC50 of less than 0.5 µM against planktonically grown bacteria and they rapidly depolarise bacterial cells to kill approximately 50% of cells within 10 minutes following treatment with 5 µM aptabiotic. Aptabiotics can dissociate both immature and mature biofilms following a single 5 µM treatment, reducing the biomass by up to 88% compared to the untreated control. A reduction in the number of viable bacteria within the biofilm and a lag in growth re-establishment time after antimicrobial challenge compared to control was also seen.

Discussion

Our findings indicate that aptabiotics are an effective novel antimicrobial agent. The rapid onset of killing occurs independently of bacterial replication, as such, aptabiotics may be effective for treating non-replicative and slow-dividing cells such as persister cells that cause recalcitrance of disease and lead to antibiotic tolerance and resistance. Our initial studies indicate that potential resistance to aptabiotics may not occur as silver ions have multiple mechanisms of killing, and aptabiotics are not substrates of MDR efflux pumps. Aptabiotics also have potential synergistic effects with common antibiotics allowing

the repurposing of antibiotics once thought ineffective due to acquired resistance.

Enhancing Aptamer Selection for Biomedical Applications

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Introduction

Aptamers are single stranded DNA or RNA molecules that are selected from a large combinatorial library of candidates to bind a target molecule (ligand) with high affinity. Aptamers have been likened to chemically designed antibodies as they are able to function in many of the biomedical applications that antibodies are typically used in. They have many advantages over antibodies in biomedical applications, most notably being synthetic they do not need to be isolated from living organisms and can be readily synthesised with modifications for specific purposes. The selection of high affinity aptamers for use in therapeutic and diagnostic applications is however, challenging and often fails because of the technical hurdles and the large number of sequence variants (approximately 100,000,000,000,000) that must be screened to find a good aptamer candidate.

We reasoned that a convolutional neural network (CNN) trained on binding data might have the capacity to select DNA sequences with enhanced ligand binding using fewer experimental steps. We sought to test this by designing aptamers to the bacterial virulence factor pyoverdine (PYO) which is secreted by the opportunistic pathogen *Pseudomonas aeruginosa* to facilitate growth and infection. In particular, *P. aeruginosa* causes significant morbidity by instigating chronic drug resistant infections in the lungs of cystic fibrosis patients

and also in diabetic ulcers; as such an aptamer to PYO would have both therapeutic and diagnostic value.

Aim

In this project we sought to compare a traditional aptamer selection strategy using SELEX (systematic evolution of ligands by exponential enrichment) with an *in silico* approach the used a high-density DNA microarray to train a neural network to investigate whether a machine learning approach can be used to enhance selection of aptamer candidates.

1. We sought to determine whether using a rationally designed pseudo-random aptamer library patterned for stable secondary structures would show discriminatory binding to PYO in under 5 cycles of SELEX.
2. To design a high density microarray containing oligonucleotides selected for stable secondary structure and evaluate this to determine whether discriminatory ligand binding at features can be resolved and quantified to form a data training set for a CNN.
3. If a CNN trained on ligand binding data can be used to predict aptamer sequences that will show increased binding affinity.

Methods

PYO was purified from *P. aeruginosa* and characterised by mass spectroscopy prior to chemically coupled to agarose beads using an epoxy linkage. The coupled beads were used as an affinity matrix for selection of aptamer candidates during SELEX rounds. Negative control agarose beads that did not have PYO chemically linked were also prepared for negative-selection rounds of SELEX. The library was designed with an algorithm to facilitate stable secondary structure while maintaining a high level of sequence diversity. This approach favours aptamer selection by reducing the complexity of the search space by eliminating sequences that are unlikely to fold into viable aptamers. Sequential SELEX rounds were performed by binding the library to the affinity column then eluting bound aptamer

candidates using higher stringency conditions. Negative selection steps using uncoupled agarose beads were performed to reduce non-specific interactions with the column matrix. Three positive selection rounds were undertaken, and the affinity of the final round was compared to the initial starting round using quantitative PCR to assess library capture.

A microarray containing 20,000 features (oligonucleotides) was designed using sequences selected from our patterned library. Each unique sequence was present in triplicate on the array. Four identical arrays were commercially prepared using a lithographic oligonucleotide synthesis procedure. Arrays were hydrated, washed in binding buffer, then heated and snap chilled to fold the oligonucleotides into their most thermodynamically stable secondary structures. Slides were then incubated with ligand for 30 min. Excess ligand was washed away and the and the retained material visualised by its intrinsic fluorescence emission at 480 nm. Images were captured with a fluorescence microscope using UV excitation with a halogen lamp, background corrected then the fluorescence intensity at each address on the array was estimated by image analysis. Two different CNN architectures were designed and evaluated.

Results

After three rounds of positive SELEX selection the affinity of the library as a whole for PYO binding was greater than the original starting material but the difference did not achieve statistical significance due to the high variation between independent experimental replicates. The library was likely enriched for tighter binding sequences, however the high sensitivity of PCR in conjunction the natural variation associated with the washing and elution steps contributed to the variation between independent experimental replicates.

Sequence specific enhancement of PYO was observed on the microarray in comparison to control sequences that were placed on the array. The control sequences were designed to have minimal secondary structure and hence were poor candidates

for functioning as aptamers. Reproducible estimates of fluorescence intensity at each element on the array could not be achieved because of rapid photobleaching of the fluorophore upon UV illumination. At the magnification level (40x) required to image each array feature, the extent of photobleaching in the 5 -10 seconds required for image focusing and capture was greater than the sequence-dependant variation in fluorescence intensity due to differential aptamer binding. Attempts to train the CNN on this noisy data were the measured effect size (sequence-dependant variation in fluorescence) was comparable to variations in repeated technical replicates due to photobleaching. Training of different CNN architectures on the PYO binding data confirmed that data set was too small relative to the measurement effect size to train the network.

Discussion

The microarray data suggest that differential ligand binding to library sequences does occur and can be detected in a library that is carefully designed to maximise the number of putative aptamers. A conventional fluorescence microscope or confocal microscope, however, is not suitable for image capture and analysis. The experiments would have likely been more successful if a microarray reader capable of imaging glass slides were available in New Zealand. Future attempts need to concentrate on reducing the variation between independent replicate measurements by enhancing the absolute amount of material bound at each feature. This could be achieved by using larger features that contain more oligonucleotide, however this approach needs to be offset against the reduced number of features such an array may contain. Increasing the signal-to-noise ratio by deep sequencing the manual SELEX rounds and synthesising the most abundant sequences on the array in conjunction with increasing the array density would likely provide a better data set for training a CNN.

Using transmission electron microscopy to asses therapeutic targets for the treatment of multiple sclerosis

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Key Findings

Multiple Sclerosis (MS) is a neurological disease affecting around 2.5 million people worldwide, and New Zealand has a particularly high prevalence. MS has symptoms including motor impairments, paralysis, pain, fatigue, muscle weakness, and visual and sensory impairments. These hallmark symptoms are due to an aberrant immune response toward the body's own Central Nervous System (CNS). This results in damage to the protective myelin coating around nerves, as well as surrounding cells in the CNS. There is no cure for MS. However, current therapies do offer limited success. Each of these therapies has a distinct mechanism of action and generally acts to suppress the immune system's attack on the myelin sheath. Unfortunately, each of these therapies has limited efficacy rates of between 30-60% for reducing relapse rates or the progression of disability.

Objectives

We aimed to evaluate new targets for an effective treatment option that functions to drive the repair of the damaged myelin sheath of the CNS using the Experimental Autoimmune Encephalomyelitis (EAE) model of MS. The objective is that these targets will complement existing therapies aimed at immune modulation. A high resolution and method of quantifying repair and remyelination in the CNS is by using Transmission Electron Microscopy (TEM). By examining images at 500-2000x magnification we are able to quantify the thickness of individual myelinated axons in a sample where a damaged axon will have little to no myelination. Our long-term goal is to use these results as a means of determining an effective treatment to cure multiple sclerosis.

Methods

Female C57/BL/6 mice aged 8-12 weeks were obtained from the Malaghan Institute of Medical Research Biomedical Research Unit (BRU) or the School of Biological Sciences SPF facility. Mice were housed at a maximum of five animals per cage. All animals are exposed to a normal 12-hour light-dark cycle and provided *ad libitum* with food and water. All handling of animals occurs in a sterile environment under a laminar hood.

EAE model: Most of the current understanding of neurodegeneration in MS comes from investigations with the EAE model of MS. This model mimics clinical features as well as the neuropathology of the disease, with immune cells entering and targeting the CNS leading to damage of the myelin sheath. Mice were immunized subcutaneously with MOG 35-55 peptide (50 µg per mouse) and an emulsified heat-inactivated Mycobacterium tuberculosis (500 µg per mouse) in the hind legs on day 0, followed by pertussis toxin (200 ng per mouse) intraperitoneally on days 0 and 2 to induce EAE. Mice are treated or receive vehicle daily by intraperitoneal injection until the experimental end point around day 40.

TEM: In spinal cord tissue from the EAE model and healthy groups, mice were perfused at a rate of 3 mL/min with 0.2% glutaraldehyde + 4% paraformaldehyde in 0.1M Sorenson's phosphate buffer. The tissue was then fixed overnight in a 2.5% glutaraldehyde + 4% paraformaldehyde in 0.1M Sorenson's phosphate buffer solution at 4oC on a rocker. Samples were processed with 1% osmium tetroxide, dehydrated, embedded in epoxy resin, and transverse sections (90-60nm) stained with uranyl acetate, lead citrate, and carbon coated for TEM imaging in the Electron microscopy suite at the MacDiarmid Institute of Victoria University of Wellington.

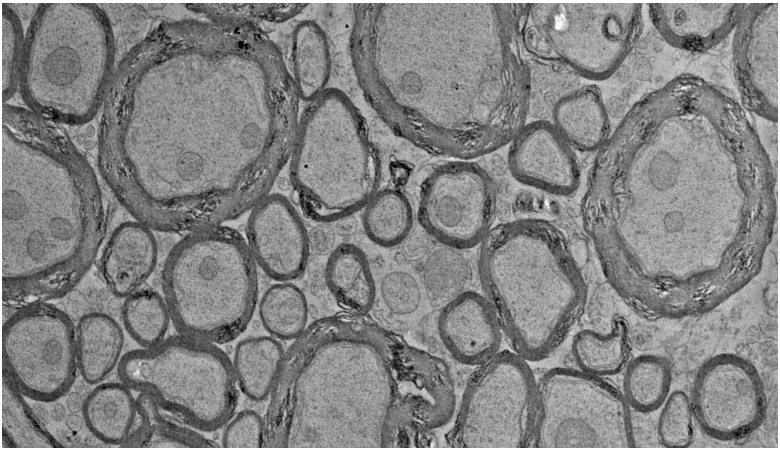
Results

Quantification by g-ratio (the ratio of the inner axonal diameter to the total outer diameter) is a highly reliable ratio for assessing axonal myelination, in addition to quantification of myelinated

vs unmyelinated axons. By collecting approximately 200 axon and axon plus myelin units from at least 3 images from each of the experimental triplicates of treatment groups, as previously defined in publications, quantification by g-ratio will allow for robust statistical evidence.

We were able to produce a reliable method for the preparation and sectioning of CNS tissue for imaging by TEM; where representative groups of healthy and vehicle treated EAE are shown in Figure 1.

A.



B.

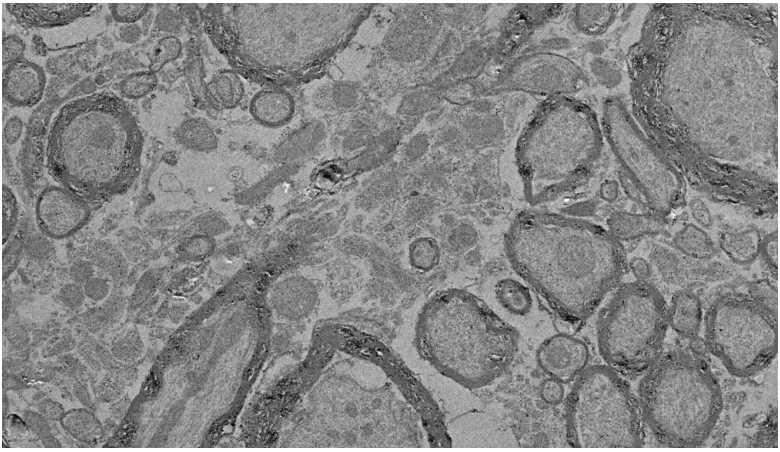


Figure 1: Representative electron microscopy images (500x magnification) of cross sections (90 – 60 nm) of spinal cord tissue isolated from A) healthy and B) vehicle EAE mice on day 40 post immunisation.

G-ratio quantification of the inner axonal diameter divided by the outer axon + myelin diameter (Figure 2) indicates a significant difference between g-ratios of healthy and EAE mice treated with a vehicle, where a higher g-ratio indicates demyelinated axons.

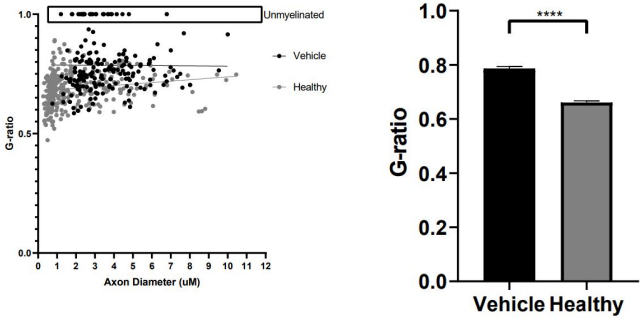


Figure 2: G-ratio quantification of spinal cords imaged by TEM at 500x magnification (n=2 vehicle, n=2 healthy). Non-parametric Mann Whitney t-test $p<0.0001$.

Conclusion

The Research For Life grant facilitated our use of the TEM at the School of Chemical and Physical Sciences in imaging biological samples, specifically CNS tissue. From this, we were able to develop a method for fixation, embedding and sectioning of CNS tissue specifically that allows for high-resolution imaging of individual axons which cannot be achieved by traditional histological methods. Continued work on the TEM will build robust results on the potential of our target treatments to remyelinate and repair CNS tissue in EAE.

MP1104, a novel mixed opioid agonist reduces chemotherapy-induced neuropathic pain in mice

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Key Findings

MP1104, a novel mixed kappa and delta opioid receptor agonist reduced neuropathic pain in male mice without producing tolerance, an effect that is a major drawback of the clinical available mu-opioid receptor pain treatments such as morphine. This is a significant unmet health problem as currently available pain medication are ineffective in treating neuropathic pain and abuse of currently available pain medications resulted in over 47,600 deaths due to overdose in the US in 2017.[1]

Objective

Our major objective was to evaluate the effectiveness of the novel mixed opioid drug MP1104 in comparison to morphine for its ability to modulate chemotherapy-induced neuropathic pain

following both acute and chronic administration, and to compare tolerance effects to those observed with morphine.

Methods

Induction of CINP: Paclitaxel (4 mg/kg i.p.) was administered on days 0, 2, 4 and 6 to give a cumulative dose of 16 mg/kg (i.p.) Using this model, induction of neuropathic pain was observed in response to both mechanical and cold stimuli. Both mechanical and cold allodynia were evaluated on alternate days up to day 15, a time where maximal stable neuropathic pain responses were observed.

Evaluation of allodynia: Allodynia is a term that refers to the triggering of a pain response from stimuli, which does not normally provoke pain. Mechanical allodynia (sensitivity to pressure) was measured using a 20-piece von Frey filament set (Stoelting, IL, USA) according to the simplified up-down method in male C57BL/6 mice.[2] Thermal allodynia to a cold stimulus was also evaluated by placing a drop of acetone on the hind paw and recording the duration of time responding to the cold stimulus using previously described methods.[3] A positive response was defined as elevating, licking, biting or shaking of the paw.

Evaluation MP1104 on CINP: On day 15, the cumulative dose-response effects of MP1104 were evaluated using a within animal design (Figure 1A). Briefly, mice were given subcutaneous injections of MP1104 at increasing doses every 1 hour to give the cumulative doses: 0.1, 0.2, 0.3, 0.5, 1, 3, 6 mg/kg/s.c. Measures of mechanical and cold allodynia were performed every 1 hour, prior to the next cumulative dose. Using these established techniques, we evaluated MP1104 alongside morphine and calculated efficacy (E_{max}) and potency (ED_{50}). Beginning on day 16 (when stable neuropathic pain is observed), mice were administered a daily dose of MP1104 at the ED_{80} dose to evaluate analgesic tolerance to both mechanical and cold allodynia.

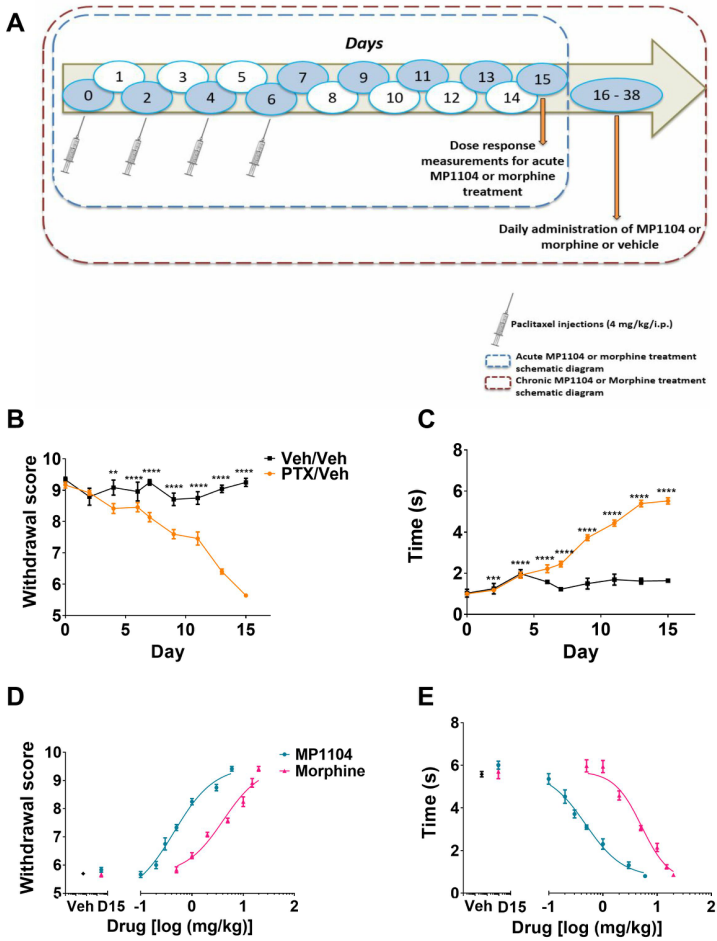

Figure 1: (A) Experimental design for acute and chronic treatment of MP1104 or the control analgesic morphine in mice with established paclitaxel-induced neuropathic pain (the shaded days indicate when animals were assessed for mechanical and cold allodynia). Paclitaxel-induced **(B)** mechanical and **(C)** cold allodynia from days 0-15 in male mice. The analgesic dose-response effects of morphine and MP1104 on **(D)** mechanical and **(E)** cold allodynia measures taken in mice with established paclitaxel-induced neuropathic pain. Veh: paclitaxel-treated mice injected with vehicle used as a control; D15: values on day 15 post-paclitaxel prior to the dose-response experiment. Values expressed as mean ± SEM, n = 6 per group.

Results

MP1104 is highly potent and efficacious at reducing chemotherapy induced neuropathic pain in mice

MP1104 attenuated both mechanical (Figure 1D) and cold (Figure 1E) allodynia in male C57BL/6 mice. Analysis of the response curves showed a significant effect of treatment in in mechanical ($F_{(4, 12)} = 11.44$, $p = 0.032$) and cold ($F_{(2, 12)} = 18.08$, $p = 0.041$) allodynia. MP1104 was around 10 fold more potent than morphine (ED_{50} values for 0.449 mg/kg and 4.07 mg/kg respectively) in mechanical allodynia ($p = 0.0386$, Student's *t*-test) and cold allodynia (MP1104, $ED_{50} = 0.479$ mg/kg; morphine, $ED_{50} = 5.179$ mg/kg; $p = 0.0371$, Student's *t*-test).

Effect on chronic administration of MP1104 on CINP

Mechanical allodynia

Chronic administration of MP1104 (1.2 mg/kg) reduced paclitaxel-induced mechanical allodynia effects on all days evaluated from days 16-38 ($p < 0.0001$). In contrast, morphine treatment (at ED_{80} dose of 10 mg/kg) showed significant analgesic effects on days 16-30 ($p < 0.0001$) (Figure 2A). Repeated measures two-way ANOVA revealed a significant effect of treatment, time and interaction of treatment and time. The main effect of treatment was compared at each time point. MP1104 reduced paclitaxel-induced mechanical allodynia effects on all days 16-38 ($p < 0.0001$), whereas morphine was only effective between days 16-30 ($p < 0.05$). From day 30 mice became tolerant to the anti-nociceptive effects of morphine, but not MP1104 (Figure 2A). Moreover, MP1104 treatment reduced the mechanical withdrawal thresholds back to those seen in healthy mice. To understand the main effect of treatment, the area under curve (AUC) was calculated for days 16-38. AUC analysis and Bonferroni post-tests showed that both MP1104 (1.2 mg/kg, i.p., daily; $p < 0.0001$) and morphine (10 mg/kg, i.p. daily; $p < 0.05$), reduced mechanical thresholds compared to vehicle/paclitaxel treated mice (Figure 2C).

Cold allodynia

MP1104 (ED_{80} = 1.2 mg/kg) and morphine (ED_{80} = 10 mg/kg) significantly reduced paclitaxel-induced cold allodynia on all treatment days 16-38 ($p < 0.0001$). There was significant effect of treatment, time and interaction of treatment and time (Two-way ANOVA with Bonferroni post-tests). Bonferroni post-tests also showed a significant effect between the vehicle/vehicle negative control and the vehicle/paclitaxel treatment on days 16-38 ($p < 0.0001$). Morphine (10 mg/kg, i.p. daily) produced a reduction in the cold stimulus response times on days 16-24 ($p < 0.05$), whereas MP1104 reduced cold allodynia at all time-points (days 16-38, $p < 0.0001$). Moreover, MP1104 treatment reduced the cold response times back to the same level as healthy mice (Figure 2B). The AUC analysis showed a significant effect of treatment ($F_{(3,24)} = 19.29$, $p < 0.0001$)(Figure 2D).

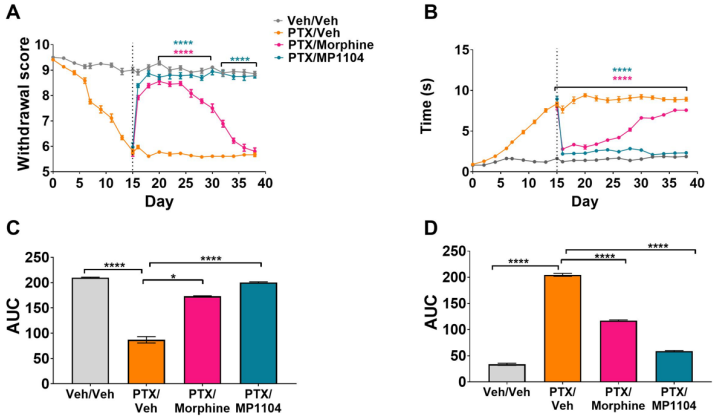

Figure 2: MP1104 (1.2 mg/kg) and morphine (10 mg/kg) daily i.p. injections reduced paclitaxel-induced **(A)** mechanical and **(B)** cold allodynia. MP1104 completely restored mechanical threshold and cold allodynia to non-diseased levels. AUC showed morphine and MP1104 reduced **(C)** mechanical and **(D)** cold allodynia compared to vehicle/paclitaxel group. Values expressed as mean ± SEM, n = 9 per group.

Conclusion

In the paclitaxel-induced neuropathic pain model, MP1104 was 10-fold more potent at reducing pain responses than morphine. Moreover, MP1104 did not exert tolerance upon repeat administration.

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Keeping intruders at bay: investigating a novel mechanism of bacterial inhibition of wound healing

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Victoria University of Wellington

Key Findings

The meningococcal virulence factor, HpuA, is well known as having a role in bacterial acquisition of iron from the host. Here we have shown that HpuA is responsible for meningococcal inhibition of wound repair, and that it does so by mediating adherence to epithelial cells, a previously unknown function of HpuA.

Objectives

Preliminary studies were done for a collection of closely related meningococcal isolates from individuals of the same household. We identified very closely related isolates that differed in their ability to inhibit epithelial wound repair. Genome sequencing

revealed that an isolate that did not inhibit wound repair only had a mutation in one gene, relative to a closely related isolate that inhibited wound repair. When this gene, HpuA, was deleted, the resulting mutant lost the ability to inhibit wound repair. HpuA is a well-studied meningococcal virulence factor that plays a role in extracting iron from the host from haptoglobin-haemoglobin complexes. The goal of our study was to determine the role that haptoglobin played in the interaction between bacteria and epithelial cells.

A review of the literature revealed that haptoglobin may be made by epithelial cells, that it has antimicrobial properties against some species of gram-negative bacteria, and that it may play a role in cell migration. Our aims were (1) to determine whether the bacteria altered the concentration of haptoglobin in cell cultures, (2) to investigate whether meningococci, either with or without HpuA, were sensitive to the antimicrobial properties of haptoglobin, and (3) to use microscopy to assess whether the different *N. meningitidis* isolates altered epithelial cell morphology or haptoglobin localisation.

Methods

Our first aim was investigated by measuring haptoglobin levels in cell culture supernatants over a time period. We used ELISAs to measure human haptoglobin, derived from the epithelial cells, and bovine haptoglobin, derived from the foetal calf serum and used by *N. meningitidis* as an iron source.

For our second aim we measured the effect of purified human haptoglobin on *N. meningitidis* growth. The bacteria were grown in the presence of haptoglobin and enumerated the following day by plating and enumerating colonies.

The third aim involved fluorescence microscopy of bacteria and epithelial cells, using antibodies against human haptoglobin. The bacteria and the host cell nuclei were stained with DAPI, and, where useful, host cell actin was also stained.

Additional experiments, expanding on the results we obtained, included quantitative assays to measure bacterial adherence (cell association assays). We enumerated intracellular bacteria using a gentamicin protection assay. HpuA was heterologously expressed in *E. coli*, and the resulting strain evaluated for adherence to epithelial cells, with or without HpuA expression.

Results

None of our results suggested any role for haptoglobin in the inhibition of wound repair. ELISA assays to determine the levels of either human or bovine haptoglobin in the cell culture medium did not identify any changes, either between the strains tested (wild type, HpuA mutant, or uninfected cells) or over a time course. This indicates that haptoglobin levels did not significantly increase in the presence of bacteria, nor did the bacteria directly or indirectly reduce the concentration of haptoglobin in suspension over 24 hours.

While haptoglobin has been reported as having antimicrobial properties against gram-negative bacteria such as *Proteus mirabilis* and *Pseudomonas aeruginosa*, we did not see any inhibition of growth or killing of *N. meningitidis*, suggesting it is inherently resistant to the antimicrobial effect of haptoglobin. This was true for both the wild type and the HpuA mutant. Similarly, the microscopy did not reveal any altered levels of intracellular haptoglobin between the isolates, and adherent bacteria were not found to be associated with intracellular haptoglobin.

The microscopy experiment did provide an unexpected result, however, as DAPI staining enabled us to see the bacteria on the surface of the cultured epithelial cells. The wild type was much more abundant on the surface of epithelial cells, relative to the HpuA mutant. We hypothesised that the surface-localised lipoprotein HpuA could have a previously unreported function as an adhesin. To investigate this possibility, we quantified the numbers of cell-associated meningococci, comparing the HpuA mutant to the wild type. A significantly reduced percentage of

the starting inoculum for the HpuA mutant was found to be associated with the epithelial cells (i.e., bacteria adhered to the surface of or inside of the cells). We quantified the invasive ability of both the wild type and the HpuA mutant and found that the mutant was significantly less invasive than the wild type.

The HpuA lipoprotein works in concert with the outer membrane protein HpuB to extract haem from haemoglobin-haptoglobin complexes. The HpuA and HpuB genes are situated in an operon on the meningococcal chromosome, and previous reports have shown that deletion of HpuA leads to loss of expression of HpuB. To determine whether HpuA or HpuB was mediating adherence to epithelial cells, we heterologously expressed Nm HpuA in a non-adherent *E. coli* expression strain, BL-21, which lacks HpuAB homologues. When HpuA expression was induced with IPTG, *E. coli* gained the ability to adhere to epithelial cells, whereas when HpuA expression was suppressed with glucose, *E. coli* did not adhere. These data confirm that HpuA is both necessary and sufficient for adherence to epithelial cells.

Conclusion

Our data suggest a novel adhesin function for HpuA. These data are consistent with a recent structural analysis of HpuA from *Neisseria gonorrhoeae* and *Neisseria meningitidis*, which identified several highly conserved residues that are dispensible binding to haem. These residues, including one exposed surface loop, were suggested to have an unidentified function apart from haemoglobin binding and haem extraction. Our future experiments will target the exposed loops in HpuA to determine which parts of the protein are involved in adherence, and we will investigate whether the HpuA from *N. gonorrhoeae* similarly acts as an adhesin. Identification of conserved residues that play a role in adherence could lead to new vaccine targets to prevent colonisation for both of these pathogens. In addition, our future

aim will be to identify the interacting human cell receptor, and to determine how its binding leads to inhibition of cell migration.

Marine natural products and synthetic analogues as potential new therapeutics [Interim report]

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Key Findings

New compounds or their derivatives may have potential for development as anticancer drugs. Production of synthetic or semisynthetic analogues of the compounds may be able to improve the selectivity for their targets and increase their potency.

Objectives

Biodiscovery is important for developing new therapeutics for treating multiple diseases. Natural products produced by plants and animals serve as a primary source of new compounds for therapeutic development. The aim of this project is to screen novel compounds and their derivatives isolated from marine animals, algae, or terrestrial plants for bioactivity against human cell lines that serve as model systems for studying disease.

Methods

Cultured cell lines are treated with compounds isolated from diverse organisms or synthesised by chemists to determine if the compounds can prevent cell proliferation and are cytotoxic to cells. The human leukaemic white blood cell line, HL-60, is used for a preliminary screen, and if activity is present in this cell line, other cancer cell lines and normal cells are tested to confirm the activity. Model cells that display the properties of other diseases can also be screened. A standard cell proliferation assay, the MTT assay, is used to assess the antiproliferative bioactivity of the unknown compound. A promising new cytotoxic or

antimitotic compound may be further analysed to determine the mode of action of the potential new therapeutic.

Results

Most of the screening at Victoria University is directed at finding new compounds that are effective at preventing cancer cell growth, although other researchers at Victoria University have looked at multiple sclerosis, anti-addictive therapy, and pain control. My laboratory has a strong interest in anti-mitotic drugs that interfere with the cytoskeleton of cells; however, new avenues of research now being carried out include investigation of sulfated sugar compounds that target the enzyme heparanase, an enzyme involved in the spread or metastasis of cancer cells. In the last year, we have identified five new classes of compounds of interest (Figure 1).

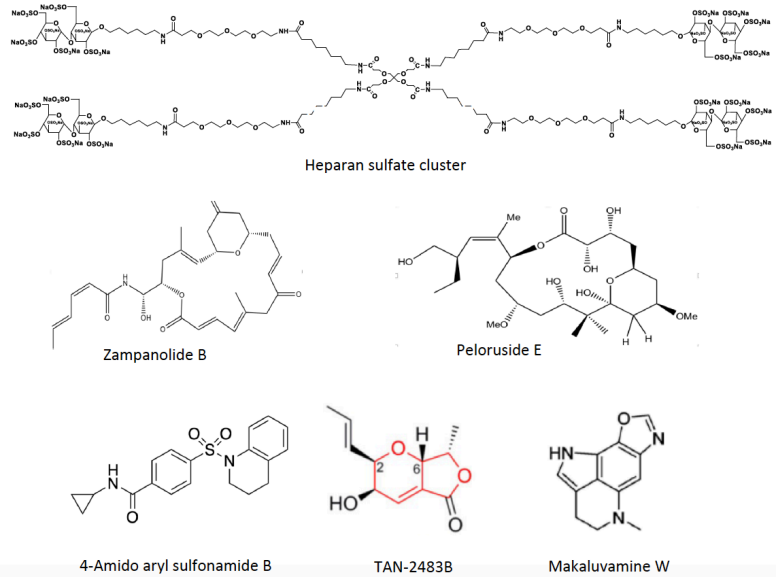

Figure 1

1) *Heparan sulfate clusters* are heparanase inhibitors that lack the anti-clotting activity of heparin-like compounds. These compounds are being tested in mouse models of breast cancer with support from the Breast Cancer Foundation of NZ and the Health Research Council. A paper describing some of this research has

recently been published: Zubkova *et al.* (2018) Dendrimer heparan sulfate glycomimetics: Potent heparanase inhibitors for anticancer therapy. ACS Chem Biol 13:3236-42.

2) *Zampanolide* and *peloruside* derivatives are microtubule-stabilising compounds with potent anti-proliferative activity. Although these compounds are cytotoxic to cancer cells at nanomolar concentrations, there is not enough material available to test them further in mouse models of cancer. We have published two recent papers on these new analogues: Taufa et al. (2018) Zampanolides B–E from the Tongan marine sponge *Cacospongia mycofijiensis* are potent cytotoxic macrolides with microtubule-stabilizing activity. J Nat Prod 81:2539-44. and Hong *et al.* (2018) Peloruside E (22-norpeloruside A), a pelorusane macrolide from the New Zealand marine sponge *Mycale hentscheli*, retains microtubule-stabilizing properties. J Nat Prod 81:2125-8.

3). *4-Amido aryl sulfonamide* has micromolar activity against cancer cells and targets the cytoskeleton. The synthesis and bioactivity of this novel compound has been published: Field *et al.* (2019) Synthesis and microtubule destabilizing activity of N-cyclopropyl-4-((3,4-dihydroquinolin-1(2H)-yl)sulfonyl)benzamide and its analogs. Chem Asian J 14: 1151-7.

4) *TAN-2483B*, a fungal metabolite synthesised by Dr Joanne Harvey, has micromolar antiproliferative activity in cells and shows anti-kinase activity. The compound has potential application for cancer treatment because of the role of certain kinases in cancer. It also prevents the resorption of bone into the bloodstream and may be helpful against osteoporosis and other bone degenerative diseases. The synthesis has been published with an image of the compound on the cover of the journal: Somarathne *et al.* (2019) Synthesis of bioactive side-chain analogues of TAN-2483B. Chem Asian J 14:1230-7.

5). *Makaluvamine W* is a *pyrroloiminoquinone* derivative that lacks an intact iminoquinone group and has lost the parent compound's activity against cancer cells. The pyrroloiminoquinones target the enzymes topoisomerase I and II. This work is published with an image of the compounds on the cover of the journal: Taufa et al. (2019) Pyrroloiminoquinone derivatives from a Tongan specimen of the marine sponge *Strongylodesma tongaensis*. Tetrahedron Lett (In press).

Conclusion

The search for new compounds from natural sources that are effective against cancer will continue to be pursued despite the very poor success rate. The rewards for developing an effective anticancer drug with good potency and few side effects would make it all worthwhile. The plant product paclitaxel is an example of a very successful anticancer drug, despite having significant side effects that include hypersensitivity, peripheral

neuropathy, and development of multidrug resistance. Numerous different formulations and delivery systems have been tried with some success, but a new compound with fewer side effects would be a great advance.

Resistance is futile: investigating collateral sensitivity to combat antibiotic resistance

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Key Findings

Microbes that have evolved resistance to one antibiotic show complicated patterns of resistance and sensitivity to other antibiotics. We have developed a new method to detect these patterns quickly and reproducibly; however, the high throughput of our approach trades off with accuracy.

Objectives

As pathogenic bacteria evolve resistance to one antibiotic, they can sometimes become susceptible to others. This phenomenon – collateral sensitivity – has attracted attention for its potential to inform smarter ways to use antibiotics. If we could predict that evolution of resistance towards drug X resulted in sensitivity to drugs Y and Z, then clinicians would know the most effective combinations of antibiotics for eliminating superbugs.

We have explored collateral sensitivity in the bacterium *Staphylococcus aureus*, which is a common hospital-acquired infection. In New Zealand, 8-10% of *S. aureus* disease is due to strains that have evolved to be methicillin resistant; that is, MRSA ("methicillin resistant *S. aureus*"). In 2015, over 1,100 people were infected by MRSA in New Zealand.

We previously evolved a laboratory strain of *S. aureus* that was resistant to oxacillin, which is closely related to methicillin. Here,

we have implemented and rigorously tested a high-throughput method for assessing this strain's collateral sensitivity to more than 70 other antibiotics. We have also compared the laboratory strains with seven clinical isolates of MRSA.

Methods

We examined the resistances and sensitivities of nine *S. aureus* strains towards 72 antimicrobial compounds. The nine strains were: *S. aureus* ATCC 25923 (a laboratory strain); *S. aureus* ATCC 25923evo (evolved in the laboratory to be oxacillin resistant); and the seven clinical isolates MRSA-BE, -BK, -BR, -SO, -SY, -TT and -UB. The clinical isolates were kindly provided by Prof Stephen Chambers (Director of Infectious Diseases, Christchurch Hospital) and Rosie Greenlees (Canterbury District Health Board). The 72 antimicrobial compounds were contained in Phenotype Microarray (PM) plates 11 to 13 (commercially available from Biolog Inc). These 96-well plates each contain 24 antimicrobials, present at different concentrations in four wells.

Each *S. aureus* strain was used to inoculate every well of PM11-13. After incubation for 24h at 37°C, the presence of a dye allowed bacterial growth to be scored by eye. Full growth resulted in a dark purple colour, intermediate growth was light purple, and no growth resulted in a colourless well. The entire experiment was repeated in independent duplicates (several weeks apart) to assess the reproducibility of the PM plates. In select cases, the accuracy of the PM plates was also cross-checked by correlating purple colour development with minimum inhibitory concentration (MIC), which in turn was determined using the standard method of broth microdilution [Wiegand *et al.* (2008), *Nat Protoc*, 3:163-75].

Results

We used PM plates 11-13 to rapidly assess 648 examples of antimicrobial resistance/sensitivity (9 strains $\times$ 72 antimicrobials). The antimicrobials were cell wall-acting antibiotics (16 compounds); protein synthesis inhibitors (22 compounds); inhibitors of nucleic acid synthesis

(11 compounds); repurposed cancer and anti-psychotic drugs (7 compounds); and others including antiseptics, disinfectants and metal ions (16 compounds). As shown in Figure 1, relative resistance to each antimicrobial was assessed using a growth score that ranged from 0 (completely sensitive) to 8 (maximally resistant).

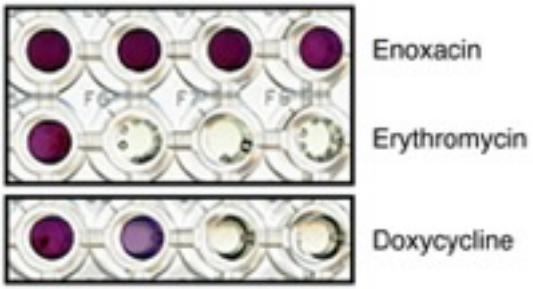

Figure 1. Examples of growth in Phenotype Microarray plates. The growth of *S. aureus* ATCC 25923 in four wells of increasing enoxacin, erythromycin and doxycycline concentrations is shown. Colour development was scored by eye as either 0 (no growth), 1 (intermediate growth) or 2 (full growth). Summing the growth in all four wells yielded a score out of 8 for each antimicrobial. In the examples shown, growth scores of 8, 2 and 3 were recorded for enoxacin, erythromycin and doxycycline, respectively.

Comparing the MRSA isolates with *S. aureus* ATCC 25923 revealed hints of collateral sensitivity; for example, the MRSA isolates all appeared more sensitive to doxycycline and 5-fluorouracil. As expected, *S. aureus* ATCC 25923 showed a low level of oxacillin resistance (average growth score = 1.5), whereas the strain evolved from it in the presence of oxacillin (25923evo) showed increased resistance (growth score = 3).

When the entire experiment was replicated, we found that the growth scores for each antimicrobial were identical 84% of the time. When the growth scores were different, it was most commonly only by one point on the 8-point scale (11.5% of cases).

We selected six antimicrobial compounds to analyse in more detail. Erythromycin and oxacillin had highly variable efficacies

against the nine *S. aureus* strains. Nickel chloride and 2,2′-dipyridyl elicited moderate variability in growth scores between strains. Cefazolin and demeclocycline had relatively uniform efficacies against all strains. In order to appraise the sensitivity and accuracy of our PM assays, we used the broth microdilution method to determine MICs for these six compounds. Our goal was to assess whether the large and small growth differences we observed in the wells of PM plates truly reflected differences in antimicrobial resistance/sensitivity.

The comparison of PM growth scores and broth microdilution MICs is shown in Table 1. In general, large differences in PM growth score reliably reflected large differences in MIC. For example, a high growth score for erythromycin (8 or 7.5) corresponded to an MIC of 128 μg/ml, whereas a low growth score (2) corresponded to MICs of 8 μg/ml or less. At the same time, the correlation between PM growth score and MIC was far from perfect. The five strains that showed a growth score of 2 in erythromycin-containing PM wells varied in their MICs from 2 μg/ml (MRSA-SO) to 8 μg/ml (MRSA-BE and MRSA-UB). Another strain with an MIC of 8 μg/ml (MRSA-SY) had a growth score of 4, not 2.

Overall, differences in PM growth score of less than 2 units could not reliably predict differences in MIC. For example, growth scores of 4, 5 or 6 could all correspond to a demeclocycline MIC of 0.0156 μg/ml (Table 1). Similarly, growth scores of 5, 7 or even 7.5 could correspond to a nickel chloride MIC of 64 μg/ml.

CLINICAL ISOLATES

MRSA isolate	Erythromycin PM score	Erythromycin MIC µg/ml	Oxacillin PM score	Oxacillin MIC µg/ml	2,2'-Dipyridyl PM score	2,2'-Dipyridyl MIC µg/ml	Nickel chloride PM score	Nickel chloride MIC µg/ml	Demeclocycline PM score	Demeclocycline MIC µg/ml	Cefazolin PM score	Cefazolin MIC µg/ml
BR	8	128	2.5	0.25	7.5	512	8	128	4	0.0313	8	2
TT	7.5	128	4.5	8	4	128	8	128	4	0.0156	8	16
SY	4	8	0	64	7.5	64	5	64	4	0.0156	8	4
SO	2	2	0	< 0.125	7.5	512	8	128	5	0.0313	5.5	< 0.125
BE	2	8	0.5	0.25	7.5	128	8	128	6.5	0.0625	8	8
UB	2	8	0	< 0.125	4	64	7	64	5	0.0156	5.5	< 0.125
BK	2	4	3	0.25	7.5	64	6	32	4	0.0313	8	2

LABORATORY STRAINS

	Erythromycin PM score	Erythromycin MIC µg/ml	Oxacillin PM score	Oxacillin MIC µg/ml	2,2'-Dipyridyl PM score	2,2'-Dipyridyl MIC µg/ml	Nickel chloride PM score	Nickel chloride MIC µg/ml	Demeclocycline PM score	Demeclocycline MIC µg/ml	Cefazolin PM score	Cefazolin MIC µg/ml
25923evo	2.5	4	3	1	5	256	7.5	128	5	0.0313	8	1
25923	2	4	1.5	< 0.125	7	32	7.5	64	6	0.0156	6.5	< 0.125

High variability compounds | Intermediate variability compounds | Low variability compounds

Table 1. Heat map to correlate PM growth scores and MICs for nine S. aureus strains and six antimicrobials. PM growth scores are shaded from dark grey (score = 8) to white (score = 0). MICs are also colour-coded, to highlight their level of agreement or disagreement with the corresponding growth score. Compounds are grouped according to the level of variability the nine strains showed in their PM growth scores.

Conclusion

PM plates are a rapid and technically straightforward way to investigate cross-resistance and collateral sensitivity, in both clinical and laboratory strains of *S. aureus.* The throughput (72 antimicrobials tested in a single experiment) is at least 3-fold greater than other published methods. Our protocol yields data that are reproducible, but that are also somewhat low-resolution. That is, PM plates are useful for identifying large differences in cross-resistance or collateral sensitivity, but they are unable to report accurately on smaller differences.

The results of this study have been submitted to *PLoS One*, as part of its Special Collection on Antimicrobial Resistance. The manuscript is currently under review.

Dissecting the genetic regulation in early phenotypes of ovarian cancer

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Key Findings

The key finding of our research were that:

1) we provide evidence that when eggs (oocytes) are lost from non-growing (primordial) ovarian follicles, the epithelial-derived somatic (granulosa) cells located adjacent to the oocyte revert to an epithelial phenotype and undergo cellular transformations into ovarian cancer (OC) tumours.
2) Sex cords and malignant adenocarcinomas have increased expression of genes involved in an epithelial phenotype, tumour progression, cellular migration and survival, and concomitantly have decreased expression of genes that are indicative of granulosa cell differentiation, genetic stability and tumour suppression.
3) The altered gene expression patterns were similar between adenocarcinomas and the precursor lesion (sex cords).

Objectives

The overall objective of our study was to identify the genes that regulate the early stages of OC in a mouse gene knock-out (KO) model that lose eggs (oocytes) prematurely. As a consequence, the ovaries undergo spontaneous pathological events that result in the development of heterogeneous ovarian tumours, similar to that observed in human OC. The predictable sequence of cellular transformation events enable prediction of the timing of the presence of precursor lesions of OC, from the age of the mice.

This study will provide information on the genetic pathways that are activated during the transition from normal tissue to the precursor lesion (sex cords) and then onto a more established tumour phenotype (adenocarcinoma). Should a selection of

activated genes in the precursor lesion be discovered, future studies may performed to provide proof-of-concept that a selection of systemic early OC stage biomarkers may be identified.

Methods

Discrete areas of tissue representing normal tissue, sex cords (precursor lesion) and adenocarcinomas were micro-dissected by laser microscopy (LCM). All ovaries were collected for LCM and the removal of correct OC phenotypes for gene expression analyses were confirmed by pre- and post-microdissection images of each tissue slice (Figure 1). The micro-dissection of normal granulosa cells from ovarian follicles of wild-type (WT) mice (Figure 1) as well as the precursor lesion (sex cords) and advanced phenotypes of OC from ovaries of *Fancd2*-KO mice (Figure 2) were collected. To ensure that the deletion of the *Fancd2* gene did not cause global changes within all tissues, neighbouring stromal cells were also collected from WT and KO ovaries. Total RNA and cDNA was prepared from tissues for gene expression quantification Genome Express Profiling System (GeXP).

The development of three gene sets (n=30 genes per set) were completed by designing primer pairs that resulted in a PCR amplicon of different sizes (i.e. nucleotide length), such that the size of every amplicon produced by each primer pair were different to each other. A chimeric primer pair was designed for each gene, combining gene-specific and universal sequences. Thus chimeric primers could be restricted and excess universal primers supplied such that all gene products were amplified with the same efficiency increasing the accuracy of the data. The differently-sized amplicons within each set were then separated by a gel capillary within the GeXP and fluorescence levels measured.

Results

Numerous genes were up-regulated in sex cords of 3-month old KO mice, and adenocarcinomas in 1-year old KO mice, compared to granulosa cells that remained associated with their oocyte in WT mice. Importantly, alterations in expression of these genes are not observed in adjacent stroma cells indicating that this was a phenotypic response. These genes included those involved in both epithelial and fallopian tube phenotype, cellular adhesion, proliferation, survival, migration and extracellular matrix organisation, transcriptional regulation of energy metabolism, blockers of apoptosis and oncogenesis. Many of the identified genes have been associated with others cancers, which showed that higher expression levels correlated with poorer outcomes.

A number of genes were also down-regulated in sex cords and adenocarcinomas in KO mice, compared to granulosa cells from ovarian follicles of WT mice. Likewise, alterations in expression of these genes are not observed in adjacent stroma cells. These genes included those involved in maintaining genomic stability and thus tumour suppression, and regulation of differentiation of follicular cells and adipocytes.

Thus, it appears that as un-regulated granulosa cells undergo cellular transformations, they revert to a more epithelial phenotype and have a tendency to over-express extracellular matrix genes involved in adhesion, migration, invasion, oncogenesis as well as lose the ability to undergo apoptotic death. In concert, they also under-express genes that maintain genetic stability and normal granulosa cell function. Interestingly, the expression of the fallopian tube marker gene was increased supporting our hypothesis that granulosa cells derived from ovarian surface epithelium, which was also derived from the coelomic epithelium, retain their propensity for potency and are capable of transforming into non-ovarian phenotypes.

Summary

This study provides novel information on the genetic pathways that are activated during the transition from normal tissue to a precursor lesion (*i.e.* sex cords) and then onto a more established tumour phenotypes (*i.e.* adenocarcinomas). Interestingly, the marked increased expression in genes involved in oncogenesis, and reduced expression in genes regulating normal granulosa cell function were prevalent in both sex cords and adenocarcinomas. The identification a specific gene signature in the precursor lesion of EOC in these mice, which was identical to the mature tumour phenotype, suggests systemic biomarkers of these early transformations may be present. These results will be prepared for publication, and they also underpin the work of a new PhD student (Sarah Sczelecki) as well as future funding grant applications.

Whilst we do not propose that women develop OC through the premature loss of oocytes, this research confirms our hypothesis that cells derived from the coelomic and then ovarian surface epithelium, which differentiate into granulosa cells under the regulation of the oocyte, revert back to an epithelial phenotype. As such, these cells retain their propensity for neoplastic transformation, and are capable of developing in non-ovarian-like tumours such as fallopian tumours.

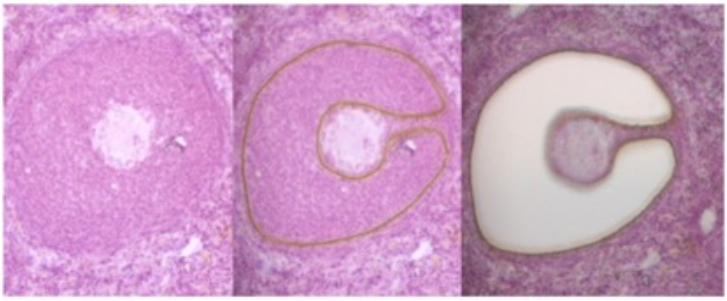

Figure 1. Laser capture microdissection of granulosa cells from a wild-type (WT) follicle
Representative micrograph of the microdissection process of granulosa cells from a one-year old FancD2 WT animal before cells are selected (left), after laser dissection (middle) and after granulosa cells were collected onto a sticky cap of the specimen tube, leaving the remaining cells intact on the specialised membrane slide (right) Images at 20x magnification.

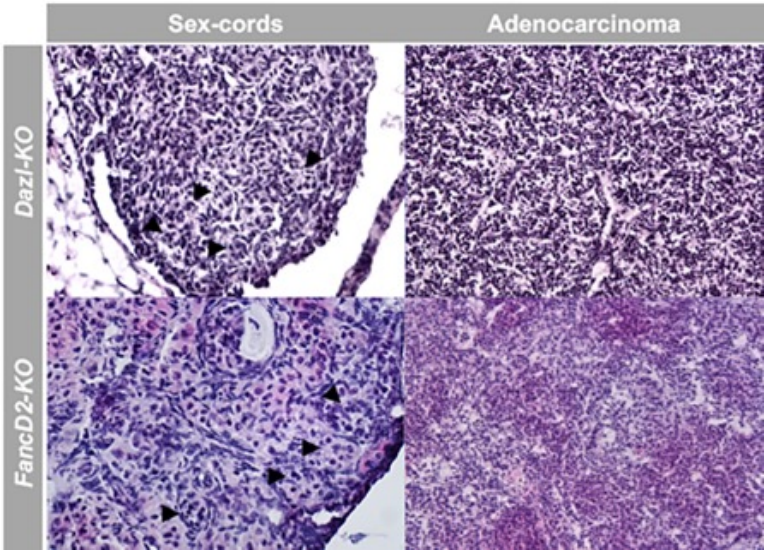

Figure 2. Micrographs of areas of epithelial ovarian cancer (EOC) targeted for laser capture microdissection from Dazl- and FancD2-knock out (KO) mice
The left-hand micrographs illustrate the precursor lesions of EOC, i.e. sec-cords (arrowheads) that consist of naïve granulosa cells devoid of the oocyte, present in young KO mice. The right-hand micrographs illustrate the histology of an advanced EOC, i.e. adenocarcinoma present in older KO mice. Sex-cord images taken at 40x magnification; adenocarcinoma images taken at 20x magnification.

University of Otago, Wellington

Measuring extracellular matrix remodelling and myocardial repair during the acute phase of myocardial infarction [Interim report]

M Brunton-O'Sullivan, A Holley, S Harding, P Larsen

Key Findings

We have completed enrolment and sample collection for 23 heart attack patients into this study. From each patient, we have collected a blood sample at baseline (less than 24 hours following a heart attack), day 2, day 3, day 4, 6 weeks and 6 months.

We have begun measurement of the 12 extracellular matrix (ECM) biomarkers at each time point to determine what changes exist across time and to identify if any patient patterns exist in ECM biomarker expression.

Objectives

The cardiac extracellular matrix (ECM) is a mechanical scaffold that provides structural support and organisation to the heart, as well as facilitating electrical transduction and cellular communication (Talman & Ruskoaho, 2016). Following a heart attack, the architecture of the ECM changes in order to compensate for the negligible regeneration capacity of the heart by replacing damaged tissue with a non-contractile scar (Frangogiannis, 2017). This process is termed ECM remodelling, and is tightly regulated by a number of molecular factors which can be measured in patient blood samples (Nielsen *et al.*, 2017). Inadequate repair following cardiac damage can predispose a patient to cardiac dysfunction and poor heart health (Zile, Gaasch, Patel, Aban, & Ahmed, 2014). Therefore, identifying patients with abnormal repair processes could provide an opportunity for targeted therapy to improve patient outcomes following a heart attack.

The objective of this study was to use a panel of ECM biomarkers to measure the magnitude and time course of the ECM response

following a heart attack. The measured biomarkers represent a number of key molecular factors associated with ECM remodelling processes, and this study will allow us to describe 1) peak ECM biomarker expression, 2) the temporal pattern in ECM biomarker levels, and 3) the relationship between changes in ECM biomarkers across time.

Methods

Patient Recruitment

We prospectively recruited 23 AMI patients presenting to Wellington Regional Hospital within 24 hours of symptom onset. Blood samples were collected from these patients at baseline (time of enrolment), Day 2, Day 3, Day 4 (if available), 6 weeks (if available) and 6 months. Baseline demographic and angiographic procedural data was recorded for all patients. This study was approved by the Health and Disabilities Ethics Committee (17/CEN/212).

ECM Biomarker Measurement

A combination of ELISA, Human Magnetic Luminex® and Cytometric Bead Array technology are being used to measure the concentration of 12 ECM biomarkers which include: matrix metalloproteinase-2 (MMP-2), MMP-3, MMP-8, MMP-9, tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), TIMP-4, periostin, osteopontin, transforming growth factor beta-1 (TGF-β1), fibroblast growth factor basic (FGFb), vascular endothelial growth factor (VEGF) and procollagen I N-terminal propeptide.

Statistical Analysis

Continuous variables were assessed for normality using the Shapiro-Wilk test. Parametric continuous variables are reported as mean ± SD and non-parametric continuous variables are reported as median (IQR). Categorical variables are reported as frequencies (percentages). All statistical analysis was performed in GraphPad Prism Software v.07 (GraphPad Software Inc; California, USA)

Results

Baseline Demographics

The baseline demographic characteristics of the patient population are shown in Table 1. The study population comprised 73.9% males with a mean age of 59 years and a mean BMI of 28.5. Of the patients in this cohort, 16 (69.6%) identified as European, three (13.0%) identified as Maori, one (4.3%) identified as Pacific Islander and a further three (13.0%) identified as 'Other'. Upon index admission, nine patients (39.1%) had hypertension, 16 patients (69.6%) had dyslipidaemia, three patients (13.0%) had prior myocardial infarction, two (8.7%) were diagnosed diabetics and eight patients (34.7%) were current smokers. Patients in this AMI cohort presented as Non-ST Segment Elevation Myocardial Infarction (30.4%) or ST Segment Elevation Myocardial Infarction (69.6%).

Table 1. Baseline Demographics of Study Population

Baseline Statistics	
Age (mean, years)	59.6 ± 10.97
Male (n, %)	17 (73.9)
BMI (mean, kg/m2)	28.49 ± 4.33
Ethnicity	
European (n, %)	16 (69.6)
Maori (n, %)	3 (13.0)
Pacific Islander (n, %)	1 (4.3)
Other (n, %)	3 (13.0)
Risk Factors	
Prior heart attack (n, %)	3 (13.0)
Hypertension (n, %)	9 (39.1)
Dyslipidaemia (n, %)	16 (69.6)
Diabetes (n, %)	2 (8.7)
Current Smoker (n, %)	8 (34.7)
Classification	
NSTEMI (n, %)	7 (30.4)
STEMI (n, %)	16 (69.6)

Continuous variables are shown as mean ± SD and categorical variables are shown as frequency (percentage). Abbreviations: BMI - body mass index, NSTEMI – non-ST elevation myocardial infarction, STEMI - ST-elevation myocardial infarction.

Angiographic Characteristics

The angiographic characteristics of the 23 study patients are presented in Table 2. Of the study population, 22 patients (95.6%) received percutaneous coronary intervention (PCI), one patient (4.3%) received medical management and a further patient (4.3%) underwent coronary artery bypass graft (CABG) surgery following PCI. The culprit lesion was identified as the right coronary artery (RCA) in 39.1% of patients, while 30.4% of patients equally presented with the culprit lesion as the left ascending artery (LAD) and circumflex artery (Cx). The median peak value of high-sensitivity troponin T (hs-TnT) was 1,189 ng/L.

Table 2. Angiographic Characteristics of Patient Population

Management Strategy	
Medical Management	1 (4.5)
PCI	22 (95.7)
CABG	1 (4.5)
Culprit Lesion	
LAD	7 (30.4)
Cx	7 (30.4)
RCA	9 (39.1)
Biochemical Measurement(s)	
Peak Troponin T (ng/L)	1189 (390 - 3774)

Continuous variables are shown as medium (IQR) and categorical variables are shown as frequency (percentage). Abbreviations: LAD - left ascending artery, Cx - circumflex artery, RCA - right coronary artery.

Blood collection

Details of blood collection are described in Table 3. The mean time from symptom onset of ischaemia to blood collection across the acute phase was 19 hours (baseline), 38 hours (Day 2), 61 hours (Day 3) and 79 hours (Day 4). Two blood samples were collected at time points remote from the index event at 6 week and 6-month follow-up appointments with a mean time of 43 days and 181 days, respectively.

Table 3. Blood Collection Measurements in patient population

Measurement	Baseline	Day 2	Day 3	Day 4*	6 weeks**	6 months
Patients (n)	23	23	23	8	17	23
Time from Symptom Onset (hours)	19.0 ± 7.2	38.0 ± 8.7	61.7 ± 8.3	79.8 ± 14.7	1022 ± 164.2	4339 ± 1031

Continuous variables are shown as mean ± SD. *Note that the majority of patients were discharged on day 3 following admission, so were not available for a day 4 measurement. **Not all patients received 6-week follow-up appointments.

Conclusion

We are in the process of measuring 12 ECM biomarkers for each of the 23 patient plasma samples. Following this we will undertake an in-depth analysis to assess what changes exist across time and to identify if any potential patient ECM biomarker patterns exist in this cohort. This information can then be applied to a subsequent future study which will examine the relationship between temporal patterns of ECM biomarkers and infarct size and acute myocardial healing.

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Zile, M. R., Gaasch, W. H., Patel, K., Aban, I. B., & Ahmed, A. (2014). Adverse left ventricular remodeling in community-dwelling older adults predicts incident heart failure and mortality. *JACC Heart Fail, 2*(5), 512-522. doi:10.1016/j.jchf.2014.03.016

Extracellular vesicles as biomarkers for disease

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Key findings

Funding was used for the purchase of an ultraspeed centrifuge (ultracentrifuge) for research purposes. The equipment has been used for the isolation and purification of sub-cellular particles for biomarker studies in patient blood and tissue samples.

Objectives

This grant-in-aid was for partial funding of a Sorvall WX 80+ Ultraspeed Centrifuge (ultracentrifuge). Previously, there was no functioning ultracentrifuge available at the University of Otago Wellington campus, Capital & Coast District Health Board (CCDHB), or Massey University College of Health. This equipment is used for the isolation and investigation of sub-cellular particles including extracellular vesicles (EVs) and bacterial membrane particles.

Results

The equipment applied for has been purchased and installed at the University of Otago Wellington laboratories. It has been used for biomedical research that has resulted in scientific conference presentations (Australasian Extracellular Vesicle Meeting, Sydney, Nov 2018; Hub for Extracellular Vesicle Investigation Symposium, June 2019) and journal manuscripts that are currently in preparation. This equipment has been used by researchers from University of Otago Wellington, Massey University College of Health, and Environmental Science and Research. It is currently being used several times per week by graduate research students for their projects. This has included projects investigating EVs as biomarkers in colorectal cancer, cardiovascular and metabolic disease, and in exercise.
The ultracentrifuge is critical for isolation of these extracellular vesicles from patient blood samples for study. Specific findings from this work include a stage-dependent increase in the concentration of extracellular vesicles in the plasma from patients with colorectal cancer versus healthy controls, and the discovery of an RNA species that is upregulated in these vesicles.

Conclusions

The purchase and installation of the ultracentrifuge at the University of Otago Wellington laboratories has increased our capacity to perform high quality biomedical research with gold standard techniques.

The role of renal bicarbonate handling in the development of acute mountain sickness

J-L Fan

University of Otago, Wellington

Background

Acute mountain sickness (AMS) occurs following rapid ascent to high altitude and affects thousands of New Zealanders who travel the Himalayas each year. Early detection could dramatically reduce the incidence and severity of AMS for those at risk. Despite over a century of research, the underlying mechanism responsible for AMS development remains elusive. Since the AMS symptoms are primarily neurological (i.e. headaches, nausea, dizziness), research efforts over the past two decades have focused on the brain's response to high altitude. But since our body's adaptive responses to hypoxia is a complex interplay between the kidneys, blood pH disturbance and the brain, a more integrative methodological approach is needed to understand the complex pathogenesis of AMS.

Objective

The objective of the present study is to examine the role of changes in kidney function in the development of AMS. Specifically, we set out to test the hypothesis that impaired kidney function at high altitude leads to greater pH imbalance and altered brain blood flow control, resulting in more severe AMS symptoms.

Methods

Using the Global Energetic and Environmental Simulation Suite (GEnESiS) at the Centre for Translational Physiology at the University of Otago, Wellington, we exposed twenty-eight healthy individuals to prolonged (up to ten hours) simulated high altitude (5000 m above sea-level). To control for any confounding circadian rhythm effects, the participants also underwent a control, ten-hour exposure to sea-level inside the GEnESiS facility. In total, 560 hours of testing were carried out

inside the GEnESiS facility, which consisted of 672 blood and urine samples and 1344 ultrasound measurements of blood flow to the brain.

Findings

We found the changes in blood pH and brain blood flow predicted AMS severity. In particular, large increases in blood pH and brain blood flow resulted in more severe AMS symptoms. These findings support the role of insufficient compensation by the kidneys to restore blood pH and subsequent over perfusion of the brain in the development of AMS.

Conclusion

We are currently in the process of preparing three manuscripts for publication to peer-reviewed journals such as *High Altitude Medicine and Biology* and *PLoS one*. We expect to submit the first manuscript in October 2019. The study was the thesis project for Ms Holly Barclay, currently a 5th year medical student, who undertook her Biomedical Science Honours degree. Ms Barclay was recently given a grade of first-class Honours for her work on this study.

Examination of the temporal variance in neutrophil phenotypes across the acute phase of a myocardial infarction

A Holley, K Hally, S Harding, P Larsen

University of Otago, Wellington

Key Findings

Patients presenting with an acute myocardial infarction (AMI), commonly known as a heart attack, display a large variation in levels of inflammatory cytokines. Analysis of these cytokines demonstrated that a more pro-inflammatory state exists earlier in the AMI, with a more anti-inflammatory state appearing later in the acute phase.

A distinct neutrophil population termed 'low density neutrophils' have been identified in people with AMI and appear to be upregulated when compared to healthy people. The current analysis is focussed on whether this neutrophil population correlates with a pro- or anti-inflammatory state in AMI patients.

Objective

The objective of this study was to assess the variation in the inflammatory response of AMI patients, and determine if a subset of AMI patients experienced a dysregulated inflammatory response.

The aim was to assess the variation that may exist in neutrophil phenotypes across the acute phase of an MI, along with assessing the cytokine profiles as a means to determine the inflammatory state of patients.

Methods

A cohort of 50 patients presenting to Wellington Regional Hospital diagnosed with an acute myocardial infarction (AMI; commonly known as a heart attack) have been recruited into this study. Inclusion criteria included hospital presentation as close to ischemia onset as possible (defined as <24 hrs since

symptom onset). Blood samples were collected at the time of study enrolment, and again daily until time of hospital discharge (average 3-4 days).

The inflammatory profile of these patients was characterised by measurement of a panel of cytokines over the acute time period. Cytometric bead arrays quantifying the circulating plasma level of inflammatory cytokines was used. The cytokine profile will be correlated to neutrophil phenotype changes over the course of the AMI, and together, they cytokine and neutrophil data will be used to determine whether a patient is displaying a phenotype consistent with a prolonged or elevated inflammatory profile.

Neutrophil phenotypes were examined using flow cytometry where we first identified them by their characteristic forward and side scatter properties. Further identification using a specific panel of cell surface markers were used to define the neutrophil populations. Changes in neutrophil phenotypes were quantified using median fluorescent intensity (MFI) of the cell surface markers.

Results

Preliminary results from measurement of inflammatory cytokines suggest that AMI patients display a huge variance in each cytokine. However, early analysis of IL-6 (pro-inflammatory marker) and IL-8 (anti-inflammatory marker) suggest that patients exhibit a more pro-inflammatory state early in the AMI that shifts to an anti-inflammatory state in the later phase (Figure 1). Statistical modelling is currently being undertaken to determine whether a subset of patients experience a prolonged or elevated inflammatory state as determined by the variance in these cytokine measures.

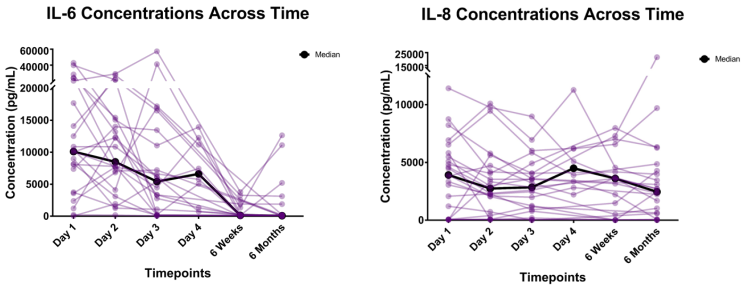

Figure 1. Levels of inflammatory cytokines across the acute phase of AMI. Peak levels of IL-6 (left) suggest that patients experience a more pro-inflammatory state early in an AMI (black line represents median), while peak levels of IL-8 (right) suggest patients experience a more anti-inflammatory state later in the AMI.

Neutrophils were found to exist in two density fractions. The majority sit in the 'normal density fraction' (NDN) with AMI patients found to have greater abundance than healthy people (Figure 2). Additionally, there is a distinct neutrophil population identified as 'low density neutrophils' (LDN) and is also found to be more abundant in AMI patients than healthy people (Figure 3). This particular neutrophil population is of considerable interest and was an unexpected find in cardiovascular patients. This population has been previously identified in cancer and other sterile inflammatory diseases, with postulation that they have an "anti-inflammatory" functional significance.

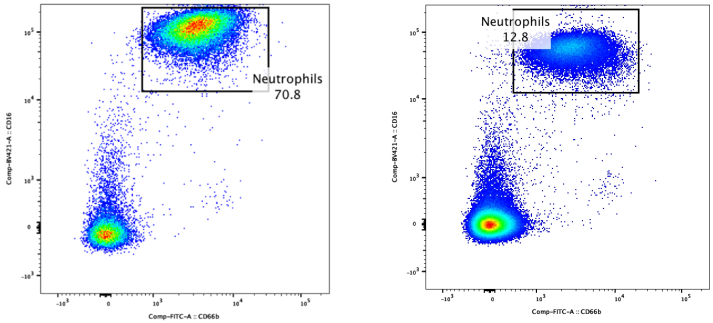

Figure 2. Normal density neutrophils (NDNs) in an AMI patient (left) vs healthy person (right). Flow cytometry plots displaying NDNs identified by side scatter properties and further characterised by CD16 (y axis) and CD66b (x axis) positivity. In the AMI patient NDNs were identified at 70.8% of the population, whereas in a healthy person they are identified as 12.8% of the population.

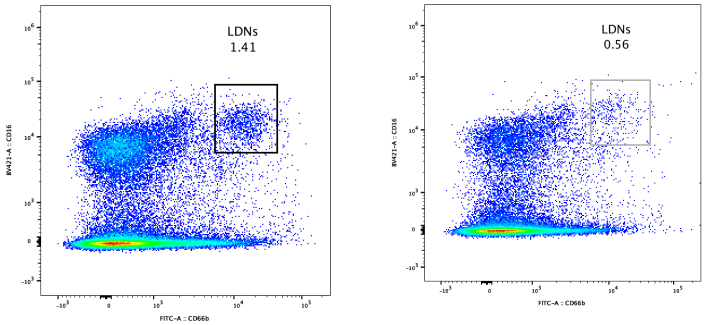

Figure 3. Low density neutrophils (LDNs) in an AMI patient (left) vs healthy person (right). Flow cytometry plots displaying LDNs identified by side scatter properties and further characterised by CD16 (y axis) and CD66b (x axis) positivity. In the AMI patient LDNs were identified at 1.21% of the population, whereas in a healthy person they are identified as 0.56% of the population.

Presently we have analysed a subset of markers from the flow cytometry panel in a subset of AMI patients. Neutrophil activation markers, CD11b, CD66b, and CD206 show a degree of variance in AMI patients over the course of hospital admission. Full data set analysis will indicate whether this variance is significant. We aim to analyse the full flow cytometry panel in the remaining 47 patients shortly.

Exciting preliminary and novel results have led to this research being expanded to a PhD project. The study cohort has been fully recruited and samples are being batch-analysed by the PhD student who commenced study at the beginning of 2019.

Conclusion

Preliminary results show that a distinct neutrophil population (LDNs) can be identified in AMI patients that have been previously described as having functional anti-inflammatory significance. Whether this LDN population displays a variance over the acute phase of an AMI will be examined fully in the banked samples. How this LDN population correlates to the inflammatory cytokine profile will also be examined. Additionally, the temporal variance of the neutrophil phenotype will be analysed by the full flow cytometry panel and correlated with the inflammatory cytokine data to determine whether a subset of AMI patients display a dysregulated inflammatory response.

Project and Travel Grants 2019

The following research and travel grants were approved for funding and will be reported on in subsequent Research Reviews.

Research

Dr Christina Baggott

What matters to people about their asthma treatments?

Asthma is a significant problem and affects over 10% of people in New Zealand. With more different types of asthma treatment regimens becoming available, Dr Baggott's research is aiming to understand what matters most to people about their asthma treatments. Future treatments can be designed with these findings in mind, and current treatments can be used more appropriately leading to improvements in how patients' asthma is managed. Dr Baggott is a specialist respiratory medicine doctor and is currently undertaking a PhD in asthma at the Medical Research Institute of New Zealand and Victoria University of Wellington.

Dr Davide Comoletti

Why is Reelin signalling dependent on its oligomerization?

The grant is to study reelin, a protein which is key to brain development and for which mutations have been implicated in autism and schizophrenia. Because of the complexity of the reelin structure and its function, Dr Comoletti's team plan to study how reelin binds to its known receptors to activate them. Dr Comoletti is a senior lecturer in the School of Biological Sciences at Victoria University of Wellington.

Dr Lisa Connor

Identifying the Molecular Messages Shared Between Dendritic Cells and T Cells

The grant is to investigate the molecular cues shared between immune cells during the initiation of an allergic immune response. New Zealand has one of the highest rates of allergic diseases in the world, affecting up to 20% of the population. The immunological mechanisms involved when an individual becomes allergic to an allergen are not well understood. Dendritic cells are master regulators of the adaptive immune system and provide the signals required to drive specific immune responses. The goal of this project is to develop a screening strategy to identify novel interactions between Dendritic cells and T cells (the cell population responsible for the symptoms of allergic disease) hoping to identify new targets for immunotherapies to stop allergic symptoms. Dr Connor is a lecturer at the School of Biological Sciences at Victoria University of Wellington.

Dr Darren Day

Does serotonin alter intracellular signalling in an animal model of depression?

The grant is to investigate whether altered serotonin uptake leads to changes in how serotonin modifies the activity of enzymes that control how connections between neurons are made and broken. Depression and anxiety are a significant social problem that arise from both genetic and environmental influences that alter the way the brain responds to the neurotransmitter serotonin. Medications for treating depression often selectively target the serotonin transporter to prevent the reuptake of serotonin, and hence are known as selective serotonin reuptake inhibitors (SSRIs). There are natural human variations in the DNA sequence that controls how much of the serotonin transporter we have in the brain, which in turn influences the likelihood of suffering from depression. Using a

genetic animal model of depression Dr Day will examine cells for changes in how key proteins involved in making connections are altered. Dr Day is a senior lecturer in the School of Biological Sciences at Victoria University of Wellington.

Dr Brendan Desmond

EV-microRNAs as biomarkers in early stage colorectal cancer

The grant is to undertake research into 'liquid biopsies', or blood tests which may aid in the earlier detection of bowel cancer. Bowel cancer is the second most common cancer in New Zealand with over 3000 new cases diagnosed and approximately 1200 deaths annually. Early diagnosis is key for reducing deaths; five-year survival for stage I and II disease is 90%, which drops to approximately 10% for Stage IV disease. Many people may not have symptoms until they have more advanced disease. Blood tests which can help detect bowel cancer early may help more people survive the disease.

Working in collaboration with the Wellington Surgical Cancer Research Group, Dr Desmond's research aims to investigate expression of microRNA in both cancer tissue and blood to determine their use as 'liquid biopsy' for bowel cancer. Dr Desmond is a Research Fellow in Surgery at the Department of Surgery and Anaesthesia, University of Otago, Wellington.

Dr Joanna MacKichan

Lethal injection: how a bloodborne bacterial pathogen avoids immune activation

The grant is to examine the bacterial pathogen Bartonella Quintana which causes trench fever, a globally prevalent infection associated with poverty and homelessness. The bacteria thrive and persist for long periods in the bloodstream, evading immune clearance. B. Quintana express a specialised secretion system that injects bacterial effectors directly into host cells. Dr MacKichan and her team hypothesise that this system aids in manipulation of the immune response. Their preliminary

data support this hypothesis. This is the first study into the function of B. Quintana effectors. Dr MacKichan proposes to characterise two key effectors and understand the mechanisms by which they hamper the host immune response to a bloodstream pathogen. Dr MacKichan is a senior lecturer in medical microbiology at Victoria University of Wellington.

Dr Johannes Mayer

High dimensional spectral flowcytometric characterisation of intestinal immune cell populations during helminth infection

The grant is to undertake research into intestinal parasite infections. Around 1.5 billion people are affected by parasite infections worldwide, with chronic infections lasting for years. However, recent studies have also shown that certain parasites can protect from more severe autoimmune and allergic diseases and Dr Mayer plans to characterise the local immune cell populations in the infected gut that might be responsible. With the help of other researchers at the Malaghan Institute of Medical Research, Wellington, Dr Mayer plans to use a novel technique of tissue preparation and high-dimensional flow cytometry to simultaneously characterise over 20 innate and adaptive intestinal immune cell populations during different stages of parasite infection. His project is among the first to study intestinal immune responses during parasite infections and will provide essential information for the development of new vaccines and immunotherapies. Dr Mayer is a Postdoctoral Research Fellow at the Malaghan Institute of Medical Research.

Dr Andrew Munkacsi

Identifying and characterizing genetic regulators of ubiquitin-dependent sphingolipid metabolism in childhood Alzheimer's disease and healthy persons

The grant is to undertake research to help children suffering with a rare disease and also provide insight into a fundamental process that is not well understood in healthy people.

Sphingolipids are critical structural and signaling molecules in all eukaryotic cells that, when defective in humans, are involved in the onset and progression of many human diseases including cardiovascular disease, diabetes, cancer and neurodegenerative diseases. However, the molecular regulation of sphingolipid metabolism is not fully understood in healthy or diseased cells.

Dr Munkacsi's research is exploring new therapeutic options to treat Niemann-Pick type C disease, a fatal paediatric neuro-degenerative disease caused by sphingolipid toxicity that typically results in loss of life before adolescence.

Terry O'Donnell

High dimensional spectral flowcytometric characterisation of intestinal immune cell populations during helminth infection

The grant is to advance research into the link between cold housing and obesity. Over a third of New Zealanders are now classified as obese and those living in deprived areas are more likely to be part of this group. Mr O'Donnell's PhD research will investigate the effect that temperature has on factors which cause obesity. In particular, it will assess whether exposure to cold temperatures may be stimulating poor eating habits.
Mr O'Donnell is a PhD candidate at the University of Otago, Wellington.

Dr Amber Parry-Strong

Understanding the utilisation of an insulin-to-protein ratio in a restricted carbohydrate environment in type 1 diabetes

The grant is to continue further two previously completed studies examining the effect of a lower carbohydrate diet (<100g per day) in people with type 1 diabetes. Many people with type 1 diabetes choose to eat lower carbohydrate as this requires them to give less insulin. However, our first study demonstrated that in the absence of carbohydrate, protein is converted to glucose and hence insulin is required for the protein content of the diet. Standardly only carbohydrate is

taken into account for insulin dosing. Our second study trialled a ratio for estimating how much insulin to give for protein in a meal test setting. This study will trial the same ratio in a free-living population under normal conditions in a real-world environment. Dr Parry-Strong is a Diabetes Research Fellow at Capital and Coast DHB.

Dr Abigail Sharrock

Engineering enzymes to protect cellular therapy vectors against activated prodrugs

The grant is to undertake research into the improvement of gene delivery vectors for gene directed enzyme prodrug therapy (GDEPT). GDEPT is a targeted anticancer strategy being developed to address the limitations of current chemotherapies including unwanted associated side-effects due to low selectivity of chemotherapeutic drugs. Dr Sharrock is a Postdoctoral Fellow in Biotechnology in Professor Ackerley's Microbial Biotechnology Research Group at Victoria University of Wellington.

Travel

Dr Naomi Brewer

Centre for Public Health Research, Massey University, Wellington

World Indigenous Cancer Conference, Calgary, Canada

Dr Brewer is an epidemiologist with research interests in cancer and inequalities. The research she will be presenting investigates the acceptability amongst Māori women of self-sampling for cervical-cancer screening. Naomi hopes that if it is acceptable in Māori and other women, this new method will be introduced in New Zealand and will increase cervical-cancer screening rates, without increasing the current disparities.

Alistair Brown

School of Biological Sciences, Victoria University of Wellington
Enzyme Engineering XXV, Canada

Alistair Brown's research focuses on as the discovery and development of novel antibiotic drug candidates. Applications of his research include high throughput screening for novel antibiotics and the development of biosensors to detect amino acids for use in both research and industry.

Dr Johannes Mayer

Malaghan Institute of Medical Research, Wellington
International Conference of Mucosal Immunology, Brisbane

Dr Mayer will present a novel technique of tissue preparation and high-dimensional flow cytometry data at the conference. With the help of other researchers at the Malaghan Institute of Medical Research, Wellington he was able to simultaneously characterise over 20 innate and adaptive intestinal immune cell populations during different stages of parasite infection, which provide essential information about anti-parasite immune responses and help the development of new vaccines and immunotherapies.

Travel Grant Reports

The following travel grant reports were received during 2019

Diana Ayupova

Computational biology of cancer conference, Paris, 2018

Applied Nanotechnology and Nanoscience International Conference, Berlin, 2018

My research involves exosomes studies and nanomaterials, to be more precise, nanoparticles (InP/ZnS quantum dots) for fluorescent labelling and biomarkers for cancer detection in a less invasive way.

In September 2018, my abstract was accepted for the first course in computational biology of cancer in Paris. This conference was devoted to computational methods for protein/gene analysis by using machine learning and other programs, such as MaxQuant. This conference opened another direction in my research project. Moreover, it brought me one step closer to proteomic analysis. It was very important for me as I am working on developing a device for cancer detection. I have developed great relationships with many speakers and possible future collaborators. The conference was conducted at Institute Pierre and Marie Curie which is one of the main centres for cancer research.

After the conference, I worked for two weeks in our collaborator's laboratory in Hospital Duran i Reynals, Barcelona, Spain, where I was learning techniques for preparing samples for proteomic analysis by using LC- MS/MS (liquid chromatography mass spectrometry) that is an exceedingly sensitive and specific analytical technique that can precisely determine the identities and concentration of compounds within the sample of interest.

The project that the proteomic unit in Barcelona works on involves nutrition studies of breast milk. For that, I had isolated exosomes from 33 breast milk samples back in my home lab in Wellington, and then brought those samples for further analysis.

My next stop was Berlin at ANNIC 2018 (Applied Nanotechnology and Nanoscience International Conference), where I presented my research on cytotoxicity (in vitro) studies of InP/ZnS quantum dots with different surface chemistries. As my future goal is developing a diagnostic device for lung cancer detection, cytotoxicity takes a major part to make sure the patient's immune system won't be compromised in any way.

This was a great experience to learn, present, and work in a wide scientific community that has the same goal to fight cancer and other disease no matter in what part of the world we are located.

Jude Ball

Youth Drinking in Decline, Krakow, Poland, 2019

Today's adolescents in New Zealand and many other high-income countries are much less likely to smoke, drink, use marijuana or be sexually active than their 1990s counterparts. This major shift in adolescent behaviour is the topic of my doctoral thesis.

When I began my PhD in 2016 the phenomenon of declining adolescent risk behaviour had received very little academic attention, and the causes were not well understood. However, over the past two- or three-years research into declining adolescent alcohol use has expanded rapidly. This burgeoning interest in adolescent drinking trends led to the Kettil Bruun Society for Social and Epidemiological Research on Alcohol (KBS) calling a thematic meeting entitled 'Youth Drinking in Decline'. The meeting was held in Krakow, Poland, April 10-12, and was jointly organised by researchers at the University of Sheffield Alcohol Research Group and the Polish National Agency for Solving Alcohol Problems (PARPA).

The meeting attracted about 60 researchers, mainly from Scandinavia, UK, Eastern Europe and Australia. The idea of KBS thematic meetings is to present and get feedback on 'work in progress'. Prior to the conference, each presenter was required

to submit a full paper, and these were made available to delegates via a password-protected website. There were no parallel sessions: all presentations were made to the full meeting in groups of two or three, and then a discussant provided constructive criticism and drew out themes for discussion.

I was one of about 25 presenters, and presented two papers, one describing trends in adolescent risk behaviours in English-speaking countries since 1990, and the other investigating potential explanatory factors for the decline in adolescent binge-drinking in New Zealand. Both were well received, but the first presentation showing parallel declines in smoking, cannabis use and sexual activity (but no improvements in mental health, obesity, physical activity or condom use) across English-speaking countries was particularly interesting to this audience, as many had an exclusive focus on alcohol and were not aware of declines in other risk behaviours.

It was my first experience of such a small and narrowly focused conference, and I found it incredibly valuable. To be in a room full of people who were curious about the same questions that fascinate me was very inspiring and affirming, and the atmosphere was collegial and supportive. Every paper was interesting and relevant to my work, but it was the qualitative studies that I got most out of. It's difficult to tell from repeat cross-sectional survey findings, on which my research is based, whether the decline in youth drinking has been imposed on young people (e.g. through lack of money or stricter parental controls) or whether they have less interest in drinking than previous generations. Qualitative findings from Australia, England, Norway, and Sweden all told a consistent story: that young non-drinkers frame their abstinence as a choice, and see drinking as irrelevant or undesirable, preferring to stay in control and prioritise other activities (e.g. sport). Other clear themes from the conference were i) a lack of evidence for the 'social media displacement' hypothesis, and ii) consistent evidence that

changing parental attitudes and practices have played a role, e.g. fewer parents hold permissive attitudes towards adolescent drinking, and parents have become less likely to supply alcohol to their adolescent children.

Kaitlin Buick

19th International Congress of Mucosal Immunology (ICMI) Brisbane, Australia, 2019

The five-day conference was centred around mucosal immunology and brought together leading experts from around the world. The conference was of particular interest as my master's thesis focuses on harnessing mucosal-associated invariant T cells to improve mucosal vaccines. With talks from experts in both the mucosal vaccine and MAIT cell fields, such as Prof. Nils Lycke and Prof. Dale Godfrey respectively, I gained insight into the current understanding and diversity of these topics, helping to drive my own research. Not only was I able to attend presentations by keynote speakers and network with a range of scientists, I was also able to present my own research during a poster session. This allowed me to communicate my work and gain valuable feedback from the other attendees. My attendance at the conference was an invaluable experience which gave me exposure to an international scientific community and has already helped to shape my upcoming experiments and future research.

Olivia Burn

CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference: Translating Science into Survival, New York, 2018

With a focus on translating science into survival the conference was well suited to the research that my PhD is focussed on. I was fortunate to listen to exceptional presentations from researchers that are trying to understand why some patients respond to treatments and others do not by looking at factors such as metabolism and the environment the tumour grows in. In addition, it was a real highlight to have one of the fellow

delegates, Professor James Allision, together with Professor Tasuku Honjo, awarded the Nobel Prize during the conference for their pioneering research on what is now one of the most successful immunotherapies for cancer.

I presented my research, 'Altering the mevalonate pathway to enhance CD8+ T cell responses', at the conference. The data I presented demonstrated a novel role of a metabolic pathway in cancer-specific immune responses. I was kept busy by numerous academics eager to learn more about these findings. These discussions were a great opportunity for me sell my research and defend the conclusions I have made from it. It was brilliant to discuss further implications of these findings with leading immunologists and listen to their advice on further experiments that would make the data more robust, and larger projects that could take these findings closer to the clinic and the patients waiting. In addition, there were many pharmaceutical company representatives who were interested in the compound my research is focussed on. Many were interested in talking to my supervisors about this and other compounds which our group researches and, if successful, these conversations will benefit our future commercialisation opportunities.

I also found the poster sessions hugely beneficial with regards to finding research groups that are working on projects that attract my interest and interacting with other early career researchers. This was a goal of my attendance and this new network will help with future employment opportunities at the completion of my PhD.

Cintya del Rio

Yeast Lipid Conference (YLC) at the Institut "Jožef Stefan", Ljubljana, Slovenia, 2019

Specialised training at University of Graz

One of the main topics of the Yeast Lipid Conference was the metabolism of sterols and phospholipids. Attending these talks helped me understand some key interactors in lipid homeostasis

that I had not thought about before. Other talks focused on lipid droplets, that is, cellular lipid storage organelles. The latter are relevant to my research because cellular density of lipid droplets has been tightly linked to the efficacy of statin treatment against tumour proliferation and metastasis, and I learned at the conference about some determinants of lipid droplet diversity, accumulation and biogenesis.

During the poster sessions I was able to discuss my results and receive input from some specialised researchers with deep knowledge in yeast lipidomics. Of particular interest were my interactions with Dr Oksana Tehlivets from the University of Graz. She was highly interested in my findings and suggested I investigate the fatty acid synthesis pathway. I have started to optimise the conditions to study the possible interactions of the essential yeast genes fatty acid synthetase fas1 and fas2 by using under- and over-expression strains of these genes.

A major benefit of my visit to the University of Graz was cementing ties with Prof Sepp Kohlwein, a leading yeast researcher, who also recently spent a short sabbatical in our lab at VUW. I also had the opportunity to be trained by the Senior Scientist Dr Heimo Wolinski to investigate lipid droplet density and characteristics using fluorescence signal of Bodipy stained-yeast cells. Further, I had the privilege to be trained by Dr Karin Athenstaedt, an invited speaker to the Yeast Lipid Conference, on a technique known as yeast tetrad dissection. This technique is used to confirm yeast mutants' phenotypes and although we have the instrument needed to apply such technique in our laboratory, we had no trained people that were able to use it. Now, I will be able to apply it and train others in our group, which I know will be of great benefit for their research.

Dr Hayley Denison

International Union against Sexually Transmitted Infections (IUSTI) Asia Pacific Sexual Health Congress, Auckland, 2018

The Congress was a joint meeting between the International Union for Sexually Transmitted Infections Asia Pacific, the New Zealand Sexual Health Society, and the Australasian Sexual Health Alliance. It was a multidisciplinary meeting combining epidemiology, public health, programme science, basic science, clinical, laboratory, behavioural research and advocacy.

The theme of the Congress was ‘He muka nō te taura whiri' which translates to 'Many Strands Make up one Rope'. The Congress brought together researchers, GPs, nurses, health promoters, educators and others working in the field of sexual health to develop strength in the sexual health workforce. It was an excellent opportunity to meet with international experts in the field and discuss the sexual health challenges we are currently facing in the Asia-Pacific region. I made contacts with keynote speakers through a ‘Lunching with the Leaders’ mentoring event, and established relationships with experts from several countries including, Australia, Canada and Hong Kong. In addition, I met with clinicians from Christchurch to discuss development of our collaborative work.

The Congress included sessions on sexuality education, transgender health, contraception, gonorrhoea and syphilis, as well as several sessions about the role of social media in sexual health, both for study recruitment as well as education. The latest STI surveillance data from New Zealand, Australia, Asia and the Pacific were disseminated, including presentations from representatives of the World Health Organisation. There was also a session about equity of access to pre-exposure prophylaxis for HIV, a topic I am very interested in.

The highlights of the meeting for me were an excellent presentation on the power of combining genomic data with epidemiologic data from Dr Deborah Williamson (Doherty

Institute, Melbourne), and Dr Carmen Logie's (University of Toronto) talk about her work with communities affected by HIV.

During the Congress, I presented results from my recent postdoctoral research, which investigated the recent trends in gonorrhoea diagnosis at the Christchurch Sexual Health Centre. This was a wonderful opportunity to present my work to an international audience and I am now working on developing this abstract into a full manuscript for journal submission.

Dr Laura Ferrer Font

34th Congress of the International Society for Advancement of Cytometry (CYTO), Vancouver, 2019

This conference is the premier international venue for showcasing state of the art advances in cytometry, and the most recent developments in the fields of flow cytometry, advanced microscopy, data evaluation and fluorescent reagents.

The meeting underlined the role different technologies play in understanding basic molecular mechanisms and human disease. I was given the opportunity to present my key findings (title of the talk: Deciding between Mass or Spectral Flow Cytometry) at the conference and to showcase our work and the advanced technology platforms housed at the Malaghan Institute of Medical Research.

Prior to the conference, Cytek held their Inaugural Aurora User Group meeting. This was a great opportunity to learn from the technology developers themselves, and also, to share experiences with people from all around the world who are using this leading technology. Also, we are planning to upgrade our 3-laser Aurora spectral cytometers to 5 laser systems by the end of the year. This is an enormous upgrade with very few labs in the world currently having this configuration. At the user group meeting I was able to meet the few people who have had this upgrade, these connections will prove very useful as we transition through this exciting and tricky upgrade later in the year.

This trip gave me focused cutting edge updates on a range of research and clinical topics utilising the power of flow cytometry. In addition, I found new potential collaborators, skills and information that will help advance all our research programmes. Overall, it was an amazing and eye-opening experience!

Kelsi Hall

Enzyme Engineering XXV, Canada

In September of this year I was fortunate enough to attend the 25th Enzyme Engineering Conference which was held in Whistler, Canada. This conference is the leading international forum for the discussion of enzyme engineering which is a central theme in my PhD research. I enjoyed listening to presentations from leading researchers in this field who I have referenced extensively in my own research. I also had to opportunity to present my poster titled "Using *E. coli* NfsA to model the limitations of stepwise mutagenesis approaches to enzyme engineering". Now that I have returned to Wellington, I am able to incorporate the new techniques and knowledge I learnt into my research.

Natalie Hammond

Yeast Lipid Conference (YLC) at the Institut "Jožef Stefan", Ljubljana, Slovenia, 2019

Specialised training at University of Graz

The Conference focused on phospholipid and sterol metabolism, protein-membrane interactions and inter-organelle membrane contact sites, and yeast as a platform for lipid production.

A big part of scientific research is networking – sharing ideas and protocols, learning what other laboratories are working on, and presenting your own research. At the YLC, I presented a poster of my research conducted over the first year of my PhD. I was able to discuss my research with a number of researchers one-on-one and gain another perspective on results gained thus far.

From these discussions, I have planned additional experiments that I hope will strengthen previous results. Therefore, attending the YLC conference has been a valuable experience, that has exposed me to what working in the research field entails.

While in Europe, I also visited Graz University. Here, I was shown techniques routinely performed in their laboratory such as neutral lipid staining with Nile Red and visualisation with a confocal microscope; and tetrad dissection – an old technique that was unfortunately lost from my current lab, but will hopefully be brought back due to these connections made at Graz University.

Sonya Hummel

AussieMit Conference, Melbourne, 2018

The Conference focused on new advances in clinical oncology, cell signalling, intracellular interactions, tools and technologies related to mitochondrial function in healthy and diseased conditions.

It was a great experience. This had been my first conference attendance and having been selected for a poster presentation was a great pleasure. Sharing my results with a specialized audience allowed me to improve my presentation skills and to gain input from a variety of research backgrounds. This has helped me to view my own research from a greater range of viewpoints and will be of great aid when writing my thesis.

The Young Investigators Symposium consisted of students and young investigators presenting their research. The talks varied from structural and applied biology to clinical studies which highlighted the importance of mitochondrial research. This was a great start to the conference as it allowed us young investigators to discuss science with one another and to share our science journeys.

I was excited that Professor Jared Rutter (Salt Lake City, USA) was speaking at this conference. He has previously used yeast to

identify mitochondrial proteomes and been appointed as HHMI investigator in 2016 for his research excellence. His talk about the identification and function of the mitochondrial pyruvate carrier was truly fascinating. Having the opportunity to discuss science with a world-class researcher has been an opportunity that I truly value. Furthermore, it allowed me to network and form connections that will be beneficial in my future academic journey.

Alvey Little

30th meeting of the American Society for Rickettsiology, Sante Fe, New Mexico, 2019

The Society of Rickettsiology is an organisation that fosters research on a wide range of vector-borne bacterial pathogens. I was lucky enough to have the opportunity to discuss my work with many accomplished researchers in my field and have returned with a veritable laundry list of ideas for how I can proceed with my work. Beyond my personal conversations, the oral presentations proved not only interesting and informative, but also broadened my understanding of the capabilities of bacteria to affect their hosts.

The poster that I presented addressed my work on *Bartonella quintana*, the causative agent of trench fever, and its subversion of the immune system by modulating host cells using a molecular syringe called a type IV secretion system to inject a protein called YopJ to suppress cellular immune activation pathways.

The conference itself took place over 4 days with a focus on rickettsial disease at the Vector-Pathogen Interface, though it included a wide range of topics for talks, ranging from the ecology of ticks, fleas, and lice, to vaccine development and the biochemistry of bacterial proteins.

Despite being an American society, presenters represented universities across the world, as well as many non-university organisations including: the CDC, French National Center for

Scientific Research, The U.S. Army Public Health Center, and the NIH National Institute of Allergy and Infectious Disease.

Nicholas Lowther

Australasian Engineers & Physical Scientists in Medicine (EPSM), Adelaide, 2018

The 2018 conference theme was science fusion. In an era of multidisciplinary teams, the blending of science, engineering and technology in medicine continues to drive innovation through diversity and creativity. Of particular interest to my research was keynote speaker Prof Loredana G. Marcu (Professor of Medical Physics, University of Oradia, Romania) who spoke on radiobiology of head and neck cancers.

My oral presentation "Individual patient dose accumulation assessing the validity of reduced PTV margin plans in head-and-neck radiotherapy" was well received. Overall, the oral presentation stimulated some discussion with medical physicists from around Australasia. In particular, medical physicists from the Auckland department were most interested. In general, this oral presentation advanced my PhD research titled *Progressing Treatment Quality in Head-and-Neck Radiation Oncology* where we are aiming to reduce the treatment related toxicity for this patient group while adding to the common knowledge and awareness of how the quality of life of head-and-neck cancer patients can be improved in New Zealand. In addition, I was awarded an Australian Institute of Nuclear Science and Engineering (AINSE) travel award as a result of receiving the highest mark awarded to a student abstract from New Zealand.

Olga Palmer

19th International Congress of Mucosal Immunology (ICMI), Brisbane, 2019

The biennial ICMI meeting focussed exclusively on the interface between the immune system and the environment of mucosal surfaces.

I presented my master's project in the form of a poster and during highly interactive poster sessions, I was able to discuss my work and receive valuable feedback from a wide range of researchers which will be useful in upcoming thesis-writing. The aim of my work is to establish an immunisation model using a synthetic peptide vaccine and compare resulting immune responses of dendritic cells (DC), T cells, and natural killer T (NKT) cells in different administration sites, such as the skin and lung.

As my first international conference, attendance provided me with an invaluable opportunity to begin building my scientific career. Being able to network and brainstorm with hundreds of other immunologists was an amazing professional development experience and an ideal way to gain exposure to what research is all about.

This year's conference included an outstanding line-up of guest speakers whose work directly relates to my research project. One of the many stand-out talks was by Prof. Nils Lycke from the University of Gothenburg, Sweden, who presented data from a recent publication about a novel antigen-specific transportation pathway that occurs independently of DCs. Listening to the guest speakers, asking questions, and establishing contacts immensely benefitted me as a first-time conference attendee.

Theresa Pankhurst

19th International Congress of Mucosal Immunology, Brisbane, 2019

I attended the 19th International Congress of Mucosal Immunology, an event that focuses exclusively on the interface between the immune system and the environment at mucosal sites. This event included an impressive line-up of invited speakers such as Dale Godfrey and Nils Lycke, who are leading scientists in their respective fields of innate-like T cells and mucosal vaccines. Participating in their seminars and others

alike, provided me with many exciting ideas to pursue moving forward with my PhD research.

The poster that I presented addressed my work on natural killer T (NKT) cells in the mucosa via intranasal vaccination with NKT cell agonist α-Galactosylceramide (α-Galcer), co-delivered with model antigen (ovalbumin) and investigating how this enhances antigen-specific antibody responses. The feedback I received during the poster sessions will be incredibly helpful moving forward with our research goal to develop safe and effective mucosal vaccines that target NKT cells as adjuvants.

Dr Xiaoyun Ren

21st International Pathogenic Neisseria Conference (IPNC), California, 2018

IPNC is a bi-annual international meeting where experts in the field of *Neisseria* pathogenesis gather from around the world to present their findings. It is also a rare meeting where basic research scientists meet with applied public health professionals to investigate the best ways to combat pathogenic *Neisseria*.

I presented a poster entitled 'Revisiting the NZ meningococcal disease epidemic using whole genome analysis'. This research was partially funded by RFL (Sept 2015). The poster was well received.

We also met with current and potential collaborators.
We discussed future research directions and project progress with our collaborators from the UK. We also met with other New Zealand *Neisseria* researchers to discuss future collaborations.
We learned about a new primary cell culture system that may be useful for dissecting mechanisms of carriage and invasion.
We also made connections with members of the Canadian and Bangladesh public health laboratories. Even though no official contracts or collaborations have been established, we will try to keep connections with the experts and establish collaborations in future.

Denise Steers

Interdisciplinary Perspectives on Intersex Conference, Lincoln University, UK, 2019

I travelled to the United Kingdom to present my PhD findings and meet with Clinical psychologist, Dr Lih-Mei Liao, based at the University College London Hospital.

People born with a variation in sex characteristics (VSC) also known as Intersex or Differences in Sex Development (DSD) face the challenge of having atypically sexed bodies. Health professionals, parents and young people must navigate complex and controversial decision-making – often ethically challenging, involving multiple decision points throughout their life, with divergent and uncertain consequences. My quantitative study recruited 10 young adults with DSD/VSC (14 to 24 years); 18 parents of children with VSC; and 22 health professionals. Using thematic analysis of interviews, we identified key themes regarding participants' experiences of decision-making. They were: communication skill, bias, understanding of diversity, psychological/peer support, bodily autonomy, disrupting norms and trust. This study was conducted in collaboration with the Intersex Trust of Aotearoa/New Zealand (ITANZ).

Meeting other researchers and Dr Lih-Mei Liao resulted in sharing of ideas and discussions for possible future collaboration to help support better health outcomes for young people born with a VSC; especially, regarding the ethical challenges around genital surgery and education for healthcare providers around being more inclusive and accepting of diversity.

At the conference my research was well received, especially for the collaboration with ITANZ. I networked with many other researchers and was pleased to be contributing to the increasing body research for people born with a VSC.

Dr Michelle Thunders

Chromatin and Epigenetics EMBO (European Molecular Biology Organisation) Workshop, Heidelberg, Germany, 2019

EMBO is an organization comprising more than 1800 leading researchers that strives to promote excellence in the life sciences. The major goals of the organization are to support researchers at all stages of their careers, stimulate the exchange of scientific information, and build an environment where scientists can achieve their best work. The workshop was held at the EMBL (European Molecular Biology Laboratory) Advanced Training Centre in Heidelberg, Germany and had a large number of internationally renowned speakers who are the leaders in the field of Epigenetics and covered the latest advances in the field, including chromatin regulation, chromatin dynamics, signalling to chromatin, nuclear architecture and dynamics, developmental epigenetics, epigenomics, epigenetics and human diseases, genome stability, environmental epigenetics, and transgenerational inheritance.

This was an amazing opportunity for me to network and to learn from the best scientists in the world in this rapidly developing field. Thanks to a research grant I received from RFL in 2017 I had some epigenetic data from the project on Myocardial Infarction which I presented as a poster at the conference. My results identified key candidate genes that are significantly differentially methylated in the higher risk group of patients who experience recurrent ischemic events. Being able to discuss my results with experts including Wolf Reik (Babraham Institute, UK), Joseph Ecker (Salk Institute for Biological Studies, USA), Geneieve Almouzni (Insitut Curie, France), Fred Winston (Harvard Medical School, USA), Anne Ferguson-Smith (University of Cambridge, UK) and Ibrahim Cisse (Massachusetts Institute of Technology, USA) amongst others was an amazing opportunity.

Part of the reasoning to attend this conference was to expand my knowledge of epigenomic methodology to include analysis of chromatin as well as DNA methylation. Being able to learn current techniques, such as CUT&RUN, as well as key issues and developments in this area from the leaders of the field such as Prof Steve Henikoff has greatly enhanced my capability to develop new research proposals as an epigenetics researcher in New Zealand.

Heidi Verhagen

Australian Society for the Study of Brain Impairment and New Zealand Rehabilitation Association Inaugural Trans-Tasman Conference: 'A Call for Action', Wellington, 2019

'A Call for Action' advanced the World Health Organisation's 2030 strategic goals to develop the global health workforce. Over 230 psychologists, physicians, various therapists, nurses and academics made up the delegation providing knowledge and sharing opportunities, as is characteristic of inter-disciplinary rehabilitation practice.

The plenary session speakers were: Jonathon Evans, Matire Harwood, Fiona Jones, Fary Khan, and Robyn Tate. They all delivered pragmatic strategies to produce meaningful research or real-life improvement to clinical services.

Professor Jonathon Evans spoke about positive psychology and how listening to music enhances engagement in people with brain injuries by increasing attention, memory and cognitive recovery.

Professor Fiona Jones told the story of a project that used a participatory design with stroke patients to co-produce a book telling their stories. The book then became part of an intervention and was used to initiate conversations to identify patients hidden needs.

Professor Fary Khan spoke about evidence-based interventions for fatigue management in neurological rehabilitation. With

humour, she encouraged people to be pragmatic, pay attention to evidence of what works, and discard interventions that waste time and resources.

Professor Robyn Tate covered outcome measurement in clinical practice. She explained a slide of single case experiment plotted data which mapped a functional measure of Steps per Day to a Goal Attainment Scale. This provided a useful tool to measure outcome variability over time with which clinicians can make evidence-based decisions about intervention effectiveness.

Various presenters produced evidence and anecdotal reports of the mental health benefits for all involved, and of engaging patients/clients in collaborative research and service design. This reciprocity resulted in not only effective translation of meaningful research but also happier and more resilient health workers.

It was disappointing that musculoskeletal rehabilitation was not well represented in any of the lectures or workshops, because musculoskeletal rehabilitation interfaces with psychological factors by motor control of movement. Movement is key to pain management and increasing participation which is an important outcome of rehabilitation. I presented my research as a poster highlighting findings that non-volitional movement is a potential treatment for chronic musculoskeletal pain. Interest in my research came from psychologists with patients who had chronic pain and very limited ranges of movement. Nobody else knew what to do with these patients and they were also at a loss.

Conference highlights were the sessions on rehabilitation consumer engagement which were instructive on how to incorporate knowledge sharing in the research design.
Dr Johnny Bourke delivered a ‘How To’ session on engaging people with lived experience of disability in spinal cord injury research. The Burwood Academy in Christchurch provides a structured process that enlists a panel of people with lived disability to review research proposals and provide suggestions

to improve study design. Dr Matire Harwood facilitated a pre-conference workshop on meeting the rights of indigenous people in rehabilitation research and service development. Delegates worked through seven discussion points to identify potential actions in research and service proposals that could be taken to address social justice issues that have arisen due to decades of colonisation. Dr Harwood promoted the idea that when you 'get it right for those who are at the bottom you get it right for everybody'. Her key message was that the benefits of relationship reciprocity are achieved by knowledge sharing whilst holding genuine regard for each other. Two examples of this process developed: the Wahakura-woven baby cots that reduced Maori infant deaths and preserved cultural practices; and the Papakura Marae Health Clinic that dramatically increased Maori engagement and trust of primary health care services.

The strong take-home message of ASSBI/NZRA 2019 entreated clinicians and researchers to listen to people with lived experience of social, health and disability challenges, then to act on their voices. Musculoskeletal rehabilitation interventions included?

Notes

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