Synthetic organogenesis of an artificial spleen

DR JONATHAN TAN

It has long been known that the spleen is a key mediator in the fight against blood-borne pathogens. Now **Dr Jonathan Tan** hopes to examine and utilise the organ's regenerative powers

What first inspired you to focus on the spleen, and how did this lead you to your current research project?

My interest in the spleen started with research I performed under the supervision of Professor Helen O'Neill at the Australian National University. During my PhD, I investigated a distinct type of immune cell that developed specifically from the spleen. Interestingly, we found that spleen stromal cells were able to produce a niche, or microenvironment, that coaxed haematopoietic stems cells into becoming this aforementioned immune cell type.

One summer, I attended the Immunology Summer Program in Japan hosted by the RIKEN Research Centre for Allergy and Immunology (RCAI), where one of the speakers was Professor Takeshi Watanabe. He was presenting research about the synthesis of artificial lymph nodes in mice and, following his lecture and subsequent discussions, we decided to form a project that combined both our interests; namely the synthesis of artificial spleen and use of synthesis technology to create and study spleen immune cell niches.

Was the Immunology Summer Program an important stepping stone for your career? Would you recommend this kind of programme to young scientists?

The RCAI International Summer Program was an incredible opportunity to learn about the latest developments in immunological research, interact face to face with highly distinguished speakers and build friendships and networks with other young scientists who will no doubt shape the future of immunological research. I was very fortunate to be one of the selected participants and I would highly recommend it to other young immunologists.

Currently in the second year of your fellowship, can you outline some of the research you have conducted thus far?

We have focused on spleen transplantations in mice and, using this, have attempted to understand how spleen tissue develops. The spleen is made up of two basic components: haematopoietic (blood) cells and stromal cells. Haematopoietic cells consist of red and white blood cells (immune cells), whilst stromal cells are often regarded as the scaffold that houses and divides blood cells into different compartments.

Stromal cells are now known to play a much larger role in immune regulation and development. Through our research, we have been able to show that the transplantation of stromal spleen cells into splenectomised (surgical removal of the spleen) mice can lead to the successful regeneration of whole spleen tissues.

What main challenges have you faced in developing your artificial synthesis techniques?

The key to synthesising immune tissue successfully is first to understand how immune tissues develop and then to mimic that process. This was precisely how artificial lymph nodes were created. However, in contrast to lymph nodes, very little is known about how the spleen develops. It is well-known that lymphoid tissue organiser (LTo) cells are required for lymph node development. By blocking the function of LTo cells in a mouse model, the generation of these lymph nodes ceases.

Interestingly, in these same mouse models, the spleen still manages to show growth and this suggests an entirely different set of cells is responsible. No group to date has been able to identify these specific cells and this was the first major hurdle in my research.

Following on from that, how closer are you to realising a viable tissue replacement therapy?

We believe we have now identified a stromal cell that directs the formation of spleen tissue; namely a spleen organiser (SPo)

cell. While this is an achievement in itself, it represents only the first step in our goal towards artificial spleen synthesis.

Further to this, we want to investigate how this cell works and interacts with other cells. Once we understand the mechanics behind SPo function, we can hopefully mimic its behaviour in artificial spleen construction. The final challenge will be in applying the technology for human therapy, a process that we envisage will take several more years at best.

Can you outline the far-reaching implications of your research in a healthcare context, specifically regarding treatment options for disorders such as cancer?

The benefit of artificial spleen construction in tissue replacement therapy is already clear. Beyond tissue reconstruction, we might find an opportunity to modify spleen structure to enhance existing blood-borne immunity.

We also know from research carried out by Watanabe that artificial lymph nodes produce unusually large amounts of antibodies. If this translates to the artificial spleen, it will provide a possible application for these engineered tissues in cancer immunotherapies.

Save our spleens

The removal of a spleen can understandably have detrimental effects on an individual's health and wellbeing, but what if it was possible to reverse these effects by replacing that spleen with an identical copy?

HISTORICALLY, THE SPLEEN has been an organ of somewhat fickle responsibilities. The Greeks and the French blamed it for melancholy, whilst the Babylonian Talmud proclaimed it was responsible for laughter. We now know that the organ, which in humans can be found somewhere between the ninth and 11th rib on the left-hand side, is part of the body's lymphatic system. Its primary function lies in the filtration of circulating blood and, on top of this, the spleen is also known to play an important role in acquired immunity, namely in the fight against blood-borne diseases.

Although it is considered a 'non-vital' organ, removal of the spleen (splenectomy) results in a number of far-reaching consequences that Dr Jonathan Tan, from the Australian National University and Kyoto University, and his team hope to negate with extensive research into artificial spleen organogenesis.

SPLENECTOMY

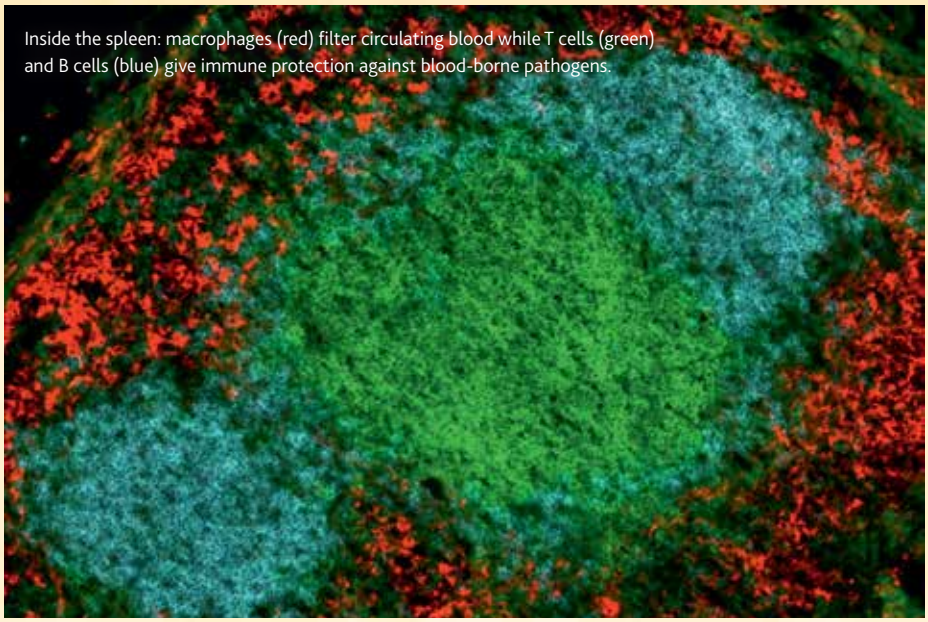
Commonly associated with trauma from motorcycle accidents, rupture of the spleen can lead to shock and, if left untreated, death. For this reason, a splenectomy is often the only option. However, whilst mortality rates are favourable, asplenic (without normal spleen function) patients are often left vulnerable to infection and dependent on prophylactic drugs. A splenectomy can also be recommended for blood disorders, such as thalassaemia, and even certain cancers meaning that the benefits to reducing the damage caused by these procedures are endless.

As mentioned, the risks that come hand-in-hand with the procedure include an increased susceptibility to infection. One case that highlighted this was a 28-year follow up on splenectomised World War II veterans, which showed a fivefold increase in mortality observed from bacterial pneumonia. Interestingly, the study also found that these asplenic individuals were significantly more likely to develop ischaemic heart disease, most probably due to postoperative hypercoagulation.

To minimise these risks associated with splenectomy, a series of protocols that include antibiotic prophylaxis and vaccinations have been put in place to try and protect asplenic individuals. With that said, these excessive treatments often come at a financial and social cost, both to the individual and the healthcare system; so for these reasons, much research has been directed towards more effective treatments including restoration of natural spleen function.

As an organ that possesses the ability to regenerate, surgeons have tried to take advantage with a relatively new technique that sees them remove part of the spleen, fragment it and then retransplant it. However, this

A TOUR OF THE RIKEN-RCAI FACILITIES, YOKOHAMA JAPAN



Inside the spleen: macrophages (red) filter circulating blood while T cells (green) and B cells (blue) give immune protection against blood-borne pathogens.

method is far from flawless, as described by Tan: "While this can be effective, the technique itself is somewhat crude and transplantation success decreases with age."

In collaboration with Professor Takeshi Watanabe at Kyoto University, Tan now wants to refine this technique in the hope that artificial spleen transplantation will become a very real treatment option following splenectomy.

STROMAL VERSUS HAEMATOPOIETIC

Cells within the spleen can be broadly grouped into two types: stromal and haematopoietic. A long line of previous evidence suggested that stromal cells alone were necessary for spleen regeneration, but surprisingly this had never being formally tested. Using simple separation techniques, Tan managed to isolate the stromal cells and show that they alone were sufficient in conferring spleen regeneration and subsequent function.

Going one step further, Tan and his colleagues hoped to identify the specific stromal cell responsible. By developing a transplant model, they were able to label specific cells with antibodies and, for the very first time, identify the cells that direct spleen formation and regeneration, which they dubbed spleen organiser (SPo) cells.

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As noted by Tan, this identification process led to a somewhat unexpected discovery: "It was quite surprising when we found that lymphoid tissue organiser cells in the spleen appear to be very different from those found in lymph nodes, as the spleen and lymph nodes are both secondary lymphoid tissues that share the same core immune functions".

Whilst these cells have now been identified, Tan hopes to investigate the cellular mechanisms involved in spleen formation and growth on a more detailed scale. One such issue they hope to examine further is the apparent lack of functional ability of SPo cells in older mice, which may offer new insight into poorer regeneration in the human adult spleen.

HEALTHCARE APPLICATIONS

This exciting identification of SPo cells has hinted at major advances within the healthcare sphere, but Tan has made sure not to get ahead of himself, fully realising that much more needs to be done before his method of treatment can become a reality. The next step lies in gaining a greater understanding of SPo cell function with regards to its interactions with other cells and its role in regeneration. Once this is done, knowledge gained can be used to artificially generate compatible spleen tissue for retransplantation.

The research's implications go even further than this, notes Tan's collaborator and mentor Watanabe: "Newly synthesised spleen tissue may provide us with a novel therapeutic strategy to fight against various pathogens and protect our body from severe infectious diseases and cancer," he explains. "More than just a replacement therapy, it could be effective even in the presence of pre-existing spleen or lymphoid tissues."

The final hurdle for their novel technique will lie in optimising the technology for human therapy, which, if successful, will revolutionise this age-old procedure. With the identification of SPo cells, the possibility of new and effective treatment options for asplenic patients is becoming a much more likely reality.

INTELLIGENCE

LYMPHOID ORGAN DEVELOPMENT: SYNTHETIC ORGANOGENESIS OF ARTIFICIAL SPLEEN AND CHARACTERISATION OF TISSUE-SPECIFIC HAEMATOPOIESIS

OBJECTIVES

- To understand which cells drive spleen organogenesis and use this knowledge to artificially construct spleen tissue
- To apply transplantation techniques developed for artificial spleen synthesis to create and study spleen-specific lymphoid niches

KEY COLLABORATORS

Professor Takeshi Watanabe, Laboratory Professor, Graduate School of Medicine, Kyoto University, Japan

Dr Yuka Kobayashi, Researcher, Graduate School of Medicine, Kyoto University, Japan

Professor Helen O'Neill, Head, Immunology and Stem Cell laboratory, Australian National University, Australia

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CONTACT

Jonathan Tan, PhD
NHMRC C J Martin Biomedical Fellow

Center for Innovation in Immunoregulative Technology and Therapeutics
Graduate School of Medicine
Kyoto University
Yoshida-Konoe machi
Sakyo-ku
Kyoto 606-8501
Japan

T +81 75 753 9511
F +81 75 753 9500
E tanj@ak.med.kyoto-u.ac.jp
E jonathan.tan@anu.edu.au

DR JONATHAN TAN completed a Bachelor of Biotechnology (BBiotech) with first-class honours and received postgraduate training in Immunology at the Australian National University. He then moved to Japan under a Japan Society for Promotion of Science (JSPS) Postdoctoral Fellowship to work in Kyoto University. Tan is currently continuing that research under a four-year NHMRC Biomedical Fellowship, returning to Australia for the final two years.

Australian National University

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