### A CD39 solution

**Dr Karen Dwyer** provides an insight into her work on improving islet transplantation as a potential cure for type 1 diabetes, outlining the key role played by CD39 and adenosine in safeguarding this tissue.

**Could you provide an explanation of islet transplantation as a potential cure for type 1 diabetes? What are the major advantages and challenges associated with this procedure?**

Type 1 diabetes results from the destruction of pancreatic beta cells and absolute insulin deficiency. At present, treatment involves administration of insulin, usually through multiple injections per day. This approach does not prevent the development of diabetic-related complications and requires the patient to be regimented in the timing and composition of their food intake.

Replacement of pancreatic beta cells is a superior approach as it recreates the normal physiological profile of insulin secretion. Although whole pancreas transplantation is one means of replacing such cells, significant morbidity accompanies this procedure. Islet transplantation is a less complicated procedure, however, challenges remain – the biggest being that multiple transplants are required. Islet cells are susceptible to the rigours of the transplant procedure and die over time. Our approach is to modify the islets prior to transplantation, thus improving their resilience.

**Can you explain current understanding of the role of CD39 in improving the outcome of this procedure, based on your research?**

The islet transplant procedure involves injecting islets directly into the bloodstream (portal circulation). This incites an acute inflammatory response causing white blood cells and blood clots to encase the islets, depriving the islet of blood supply and oxygen and thus leading to islet death. CD39 plays a role in modifying both the immune and clotting responses. We have shown that when isolated mouse islets are mixed with human blood, the blood clots within a few minutes, but if we overexpress CD39 on these islets then clotting is significantly delayed.

The advantage of targeting the expression of CD39 to the islet (as opposed to systemic administration of a CD39-like drug) is that the side-effect profile is significantly attenuated. Delivery of such an agent through a vein, for example, would lead to systemic anticoagulation with the potential for unwanted bleeding episodes. Expression on the islet itself should only prevent the clot forming around the transplanted islets. In a mouse model of islet transplantation, we have shown that the expression of CD39 improves initial engraftment, presumably through inhibiting this inflammatory process.

In addition, once islets are injected into the circulation they lodge in the liver where they start to produce insulin. Type 1 diabetes results from the autoimmune destruction of islets. Using a T cell-mediated model of diabetes, we have shown that the overexpression of CD39 on the islets significantly reduces susceptibility to diabetes, suggesting that these islets may be more resistant to the development of recurrent autoimmune diabetes.

**Does CD39 offer any protection against transplant rejection?**

We are currently investigating this, but there is literature supporting a role for adenosine in retarding the immune response. Our hypothesis is that CD39 overexpression will reduce the level of anti-rejection drugs required to prevent rejection. The knock-on effect is fewer side effects for the patient, which is a significant cause of ongoing patient morbidity following transplantation.

**What are the overall objectives of your investigations into CD39?**

The overall objectives of the investigations are to define CD39 biology and adenosine signalling in health and disease. Adenosine is produced innately by the body in response to injury as a means of stopping further damage. The majority of adenosine produced results from the actions of CD39. Giving extra adenosine to a person is possible but not effective because the half-life of adenosine is in the order of seconds, so the effect is lost almost immediately. Furthermore, adenosine has a profound effect on the heart; indeed, the one clinical use of adenosine is in the treatment of a cardiac tachyarrhythmia. ‘Delivering’ adenosine through CD39 overexpression enables a continuous supply and targeted strategy.

**How do you plan to move forward with this study?**

We now have proof of principle that CD39 overexpression protects against diabetes. However, translating this to clinical practice is not currently feasible. Therefore, we plan to administer novel adenosine-like compounds to mice to determine the effect in preventing diabetes. We will also examine the effect these drugs have on the immediate response that occurs with transplantation. Finally, we will investigate the effect of adenosine signaling on the rejection process. This will be examined firstly with CD39 overexpressing islets and subsequently with novel drugs.
AT PRESENT, THE most commonly available therapy for type 1 diabetes is the regular administration of insulin; a solution that treats but does not cure the condition. Consequently, diabetes causes significant morbidity and mortality. If the disease is to be cured and healthy insulin secretion restored, the faulty pancreatic beta cells must be replaced.

Replacement of islets can be achieved by transplantation of either the whole pancreas or the pancreatic islets. While islet transplantation is the safer and less invasive option, it does have one major limitation: the islets die off gradually following transplantation, meaning that multiple transplants must be undertaken to maintain their beneficial effects. Until scientists can identify a way of making these islets more resilient and thus suitable for transplantation, islet transplants cannot become a standard treatment for type 1 diabetes within clinical care settings.

PROLONGING ISLET SURVIVAL
Inside the laboratory of Dr Karen Dwyer, a clinician-researcher at Deakin University, work is underway to explore the potential of the human enzyme CD39 as a means of prolonging islet graft survival and function, with a view to applying these findings for the treatment of type 1 diabetes.

CD39 initially came to Dwyer’s attention due to its ability to counteract the processes that lead to the destruction of transplanted islets, via the hydrolysis of the pro-thrombotic molecule ADP and the pro-inflammatory molecule ATP. Indeed, it has been found that CD39 works in concert with CD73 to produce adenosine – which possesses anti-inflammatory and anti-thrombotic properties – as a means of protecting against the harmful effects of ATP’s release from cells following injury. If this ability is harnessed, it could potentially be used to reduce islet destruction post transplantation and so improve transplant outcomes.

MICE OF ALL TYPES
Dwyer and her team initially used CD39 knockout mice to investigate the role played by CD39 in the regulation of organ-protective adenosine receptor signalling. The mice had been generated by Dwyer’s long-term collaborator Professor Simon Robson of Harvard Medical School, USA – a world-leader in the study of CD39. Dwyer was able to demonstrate that these knockout mice were more susceptible to hypoxic injury than their wild-type counterparts, indicating that CD39 plays an important role in limiting injury. This led the team to hypothesise that increasing CD39 expression might protect against inflammation and, therefore, transplant-related injuries.

With this in mind, the team began to conduct experiments involving CD39 transgenic mice in which the enzyme was overexpressed. The overexpression of human CD39 conferred protection in experimental models of renal injury, including transplantation mediated by its role as the major immune and vascular ectonucleotidase. As one of the major obstacles to islet survival following transplantation is activation of the clotting cascade, CD39 is well poised to have impact. Indeed, CD39 overexpression on isolated islets was found to

PROTEINS WORKING IN CONCERT
Dr Karen Dwyer discusses the relationship between CD73, CD39 and adenosine

“Adenosine is generated from the sequential hydrolysis of adenosine triphosphate (ATP) by CD39 and CD73. Under normal conditions, neither CD39 nor CD73 are expressed on the islet cell itself. CD73 plays an important role in leukocyte trafficking and together with islets engineered to express CD39 will generate adenosine locally to modify the inflammatory response associated with transplantation.”

Researchers at St Vincent’s Hospital Melbourne and Deakin University, Australia, are investigating CD39 biology and adenosine signalling with a view to improving the feasibility of treating type 1 diabetes with islet transplantation.
treatment of type 1 diabetes

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The question as to whether CD39 overexpression could modify these T cell responses was answered using the multiple low-dose streptozotocin (MLDS) model of diabetes, which is T cell-dependent. The researchers found that CD39 over-expression on the islets alone conferred robust protection against recurrent diabetes mediated principally through adenosine 2A and 2B receptor signalling mechanisms. The effect on the alloimmune response at play in the rejection process is currently under investigation.

ADENOSINE ANALOGUES

As a result of this research, Dwyer and her collaborators are now working to develop clinically useful adenosine analogues; those that are currently available are severely limited by their extremely short half-life and potential to cause adverse cardiac effects such as bradycardia and hypotension. “A number of adenosine receptor analogues are available for the A1 and A2A receptors, but there is a paucity of analogues for the A2B receptor, and non-specific analogues cause a lot of side effects because adenosine receptor expression is widespread throughout the body,” Dwyer elaborates.

“As such, our studies are now looking at novel adenosine-like compounds, which are stable and only activate specific downstream signalling pathways, which produce the desired effect and eliminate the potential for unwanted side effects.”

To achieve this, the scientists are employing a paradigm known as biased agonism, in which drugs are designed that ‘bias’ specific downstream signalling pathways and in so doing selectively promote advantageous effects whilst minimising adverse ones. Many of these compounds have been developed by Dwyer’s collaborator Dr Lauren May at the Monash Institute of Pharmaceuticals (MIPS) at Monash University, Australia. Already, the researchers have identified one particular biased agonist as having potential: VCP746.

The plan at present, therefore, is to press forward with research aimed at assessing the ability of various adenosine receptor analogues to promote beta cell regeneration. The team will be studying adenosine A2AR and A2BR analogues as well as VCP746, looking at their ability to preserve islet mass under conditions of elevated metabolic demand. Specifically, there are three aspects relating to the adenosine receptor agonists that the group hopes to elucidate: their effects in models of T cell-mediated diabetes; their signalling mechanisms in beta cells in vitro; and their effects on human islet yield and function.

MOVING TOWARDS A CURE

Overall, Dwyer hopes that her efforts to elucidate CD39 biology and adenosine signalling in health and disease will ultimately lead to improved islet transplant outcomes – a result that could facilitate the treatment’s progress towards becoming the standard of care for patients with type 1 diabetes. Given that incidence of the condition is on the rise globally, any progress that can be made in this area would have far-reaching and significant applications.

**OBJECTIVES**

- To evaluate whether the overexpression of CD39 on islets improves engraftment and prolongs graft survival
- To assess the efficacy of adenosine A2AR, A2BR analogues and VCP746 in preserving islet mass under conditions of increased metabolic demand

**KEY COLLABORATORS**

Professor Simon Robson, Harvard Medical School, Boston, USA
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DR KAREN DWYER is Professor of Medicine at Deakin University, Australia. As a clinician-scientist with a focus on improving transplant outcomes for patients, her research centres on the biology of CD39 and adenosine signalling in health and disease. Dwyer’s ongoing work is examining how islet transplantation can be improved, thus making this treatment the first-line therapy for patients with type 1 diabetes.

**THE ROLE OF CD39 AND ADENOSINE RECEPTOR ANALOGUES IN IMPROVING THE OUTCOME OF ISLET TRANSPLANTS TO TREAT TYPE 1 DIABETES**

**DIABETES IN NUMBERS**

- Type 1 diabetes affects approximately 1 in 10 diabetics worldwide (34.7 million out of 347 million)

  Diabetes-related complications can include blindness, amputation and kidney failure

- Diabetes can also kill – in 2012, it was directly responsible for 1.5 million deaths

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