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# Basic sciences in development: What changes will we see in transplantation in the next five years?

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**Abbreviations**

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| BAFF | B cell activating factor |
| CADI | chronic allograft damage index |
| CAR | chimeric antigen receptor |
| cf | cell free |
| GWAS | genome wide association studies |
| iPSC | induced pluripotent stem cells |
| IRI | ischemia reperfusion injury |
| MSC | mesenchymal stem cells |
| NK | natural killer |
| PCR | polymerase chain reaction |
| TCR | T cell receptor |
| Tfh | follicular T helper |
| $T_{reg}$ | regulatory T cell |

**Abstract**

Over 3000 delegates attended the 26st International Congress of the Transplantation Society in Hong Kong, which marked the 50th anniversary of the society. In his presidential address, Professor Phil O'Connell from the Westmead Hospital in Sydney, Australia, commented that a major challenge for the future is improving long-term outcomes after solid organ transplantation. He highlighted that 40% of transplanted organs are lost within 10 years, and that a high proportion of patients suffer from immunosuppression-related side effects. These two issues are the key drivers for basic scientists in the field of transplantation. A deeper understanding of the biological mechanisms involved, and more accurate identification of patients at risk for poor outcomes or drug-related adverse effects, will ultimately help to address these problems and improve graft survival rates and patients' quality of life. The research was more diverse than ever before, reflecting the variety and complexity of the many processes which underlie outcomes after transplantation, and the need to broaden our thinking when seeking solutions to the wide-ranging complications which face the transplant team. This article explores the latest developments in basic science presented at the Congress which may offer new insights and solutions to the current challenges in organ transplantation. With so many interesting papers presented, it is impossible to discuss everything, but some key areas are considered.

**Overcoming barriers in xenotransplantation**

In the plenary session *'Transformational Sciences in Transplantation?'* Professor Joseph Tector from the University of Alabama in the United States gave a presentation entitled '*CRIPR/CAS9 – The promise of gene editing technologies in transplantation'*. According to *Science*, this technology was the major breakthrough of 2015,[1] and is expected to have the same impact as the Nobel prize-winning discovery of polymerase chain reaction (PCR) in the late 1980s.[2] Parts of the CRISPR/CAS9 system cannot easily be translated into simple terms,[1] but the crucial point is that it is a gene editing system which can knock out a gene in cells, with many potential applications. The system consists of a short segment of RNA that is attached to an enzyme. The RNA is preprogrammed to locate a unique short sequence of DNA and slice it out of the genome. It acts rapidly and is relatively precise. This technology opens up the possibility of conveniently knocking out genes involved in disease processes – and also genes that are a barrier to xenotransplantation. It has already found its way to the clinic, with one of the first registered clinical studies being undertaken by scientists at the West China Hospital in Chengdu, China, who are using the CRISPR/CAS9 system to knock out the gene encoding for immune checkpoint receptor PD-1. Activation of this receptor normally acts as a 'brake' on the ability of T cells to launch an immune response.[3] The gene-edited T cells, without PD-1, will then be expanded in the laboratory and re-introduced into the patient's bloodstream where it is hoped they will migrate into cancers to stop their growth or even decrease the tumor mass.[3] CRISPR/CAS9 could potentially revolutionize transplantation and transplantation immunology. In xenotransplantation, for example, it allows endogenous porcine retroviruses to be inactivated, avoiding the risk for retrovirus infection of human cells.[4,5] Yang and colleagues have shown that

CRISPR/CAS9 can be used to inactivate 62 active virus sequences embedded in the pig genome, resulting in a dramatic reduction of virus production.[5] This technology can also be used to genetically modify pigs to suppress the human anti-pig immune reaction, as described by Tector and his team.[6]

**Advances in immune monitoring**

There is a growing movement towards standardization of immune monitoring and biomarker studies, including standardized blood collection procedures and techniques such as flow cytometry and Elispot.[7] This is vital to avoid the problems associated with use of nonvalidated biomarkers, which are likely to have contributed to nonreproducible laboratory data over the past 10–20 years. Even small variations in cell isolation procedures, incubation times, or choice of reagents or equipment influence measurement outcomes, all of which can be minimized by using standardized procedures.

With this in mind, the talk by Matthew Albert from Genentech in the United States on next-generation immune monitoring provided a helpful update on new directions in this field. He emphasized that large-scale human immune profiling can be successful if an integrated omics approach is applied, with genome-wide association studies (GWAS) being supported by immune cell function studies. Since cell function in the adaptive immune system is heavily influenced by environmental factors, standardization of blood collection and culture procedures is essential in order to avoid wide fluctuations in T and B cell function induced by irrelevant factors. Albert stressed that standardized approaches in biomarker research are also needed during drug

development, enabling transcriptional profiling to elucidate complex-induced immune responses.[8]

There is a clear imperative to improve biomarker research. The technical developments described by Matthew Albert, and by the virtual Global Transplantation Laboratory group, will enable identification of patients at risk for graft loss, and offer an understanding of the underlying immune processes. This will provide a deeper knowledge about the mode of action of immunosuppressive drugs, the main focus of research. Results from these biomarker studies will be instrumental in developing immunosuppressive therapies tailored to the individual patient.

A glimpse of how future biomarker studies in transplantation could be designed, and the implications of their results, is given by the GoCAR study undertaken by O'Connell and collegues.[9] The authors analyzed 159 protocol renal biopsies using the affymetrix gene expression array, and divided the cohort into a discovery set and a validation set. The results demonstrated that genes associated with development of fibrosis are already highly expressed three months after transplantation in patients with a high biopsy chronic allograft damage index (CADI) score at 12 months. This was associated with early graft failure and was predictive for graft loss. Data presented by Valeria Mas from the University of Virginia in the United States on the genomics of allograft fibrosis and rejection[10] are in line with these results. Altogether, these data point to an ongoing cycle of subclinical inflammation and injury that leads to fibrosis, loss of function and graft failure. In addition, these studies demonstrate an urgent need for specialized

immunosuppression targeting fibrotic molecules and pathways, an important challenge for the coming years.

**Ischemia reperfusion injury and tissue repair**

A leading topic at the Congress was the prevention and treatment of ischemia reperfusion injury (IRI). Several papers were presented on the role of the immune system in IRI. One concerned the study by Zarrinpar and colleagues from the University of California in the United States, who demonstrated that the expression of innate and adaptive cytokines correlate with the degree of IRI.[11] The important point about this study is that it was performed in a large cohort of liver transplant patients, thereby confirming findings of previous animal studies.

Other insights have led to the development of innovative approaches and agents for treating IRI via nanoparticles, as presented by Stead et al[12] from the Royal Adelaide Hospital in Australia, and by follistatin, as shown in a study by Cowan and colleagues from St Vincent's Hospital in Melbourne, Australia.[13] For example, follistatin binds and inhibits the activity of activins, members of the TFG-ß superfamily and key drivers of fibrosis. In a mouse model of IRI, intramuscular injection of a recombinant adeno-associated viral vector carrying the follistatin gene resulted in sustained elevation of circulating follistatin levels and was associated with reduced renal expression of pro-fibrotic TFG-ß, and type I and IV collagen, with improved renal function and amelioration of IRI-induced fibrosis.

Many presentations were given by scientists from around the world about the most appropriate preservation system for donor organs, according to their quality, to achieve optimal outcomes: hypothermic or normothermic, static or perfused, continuous or final (either exclusively or possibly in combination).[14] These subjects were discussed in a commentary by Hunter and Ploeg[14] from the University of Oxford, United Kingdom, regarding a paper by Kaths and colleagues from the University of Toronto and from the Johannes Gutenberg University in Mainz, Germany[15] who reported that eight hours of continuous normothermic ex vivo kidney perfusion is a safe preservation technique that does not cause graft injury.

The subject of repairing tissue in damaged donor organs, based on the application of bioengineering skills to organ transplantation, was presented by Orlando from the Wake Forrest School of Medicine in the United States and by Hoogduijn from the Erasmus Medical Center in Rotterdam, The Netherlands. The talk by Guiseppe Orlando about *printing* organs was truly spectacular and showed that bioprinting living tissues is close to becoming reality, and is no longer science fiction. His presentation highlighted the possibilities for bioprinting and explored how it might be used to address the needs in organ transplantation by providing organs on demand without the need for immunosuppression, and potentially helping to repair injured tissues[16]. Of course, bioprinting still has its limitations but progress is being made in finding solutions for questions such as the choice of cell types and biomaterials, and relating to vascularization and innervation of the printed tissue constructs to achieve normal tissue function. The progress made over the last couple of years is most encouraging. Hoogduijn presented data on kidney regeneration through delivery of human mesenchymal stem cells (MSC), which are

known for their immunomodulatory properties.[17-19] Another solution to organ repair might be found in growing organs from human induced pluripotent stem cells (iPSC). Hoogduijn has used the protocol published by Takasato in *Nature* in 2015[20] using these iPSCs to grow kidney cells. Pilot experiments have shown remarkably complex structure formation in human iPSC-derived organoids after 18 days of differentiation. Staining on sectioned kidney organoids showed the presence of cells organized in tubule-like structures with an open lumen. In addition, q-PCR of iPSC genes demonstrated reduced expression, whereas expression of early kidney genes was strongly induced, confirming differentiation towards kidney-like cells.

Over the last few years, impressive progress has been made in the generation of insulin-producing cells. Studies by Zavazava from the University of Iowa in the United States showed that differentiated human iPSCs secrete insulin after transplantation of the organoid under the kidney capsules of diabetic immune-deficient mice. Serum glucose levels gradually declined to normal or near-normal levels over time. Furthermore, the pancreatic organoids showed neovascularization and stained positive for insulin and glucagon.[21] Others, such as scientists working in Doug Melton's team, published data showing that stem cell-derived ß-cells can be generated directly from human iPSCs and that these cells exhibit similar function to primary human ß-cells in vitro and in vivo posttransplantation.[22] These promising data show that a pancreatic organ can be created by human pluripotent stem cells and that cell treatment is a promising new option for the management of type 1 diabetes.

**The adaptive immune system: Regulatory T cells, tolerance and immune monitoring**

For many years, regulatory T cell ($T_{reg}$) therapy has attracted intense interest. Although having been a familiar topic for some time, optimized in vitro expansion protocols for regulatory T cell ($T_{reg}$) therapy have only recently been developed.[23-25] Paul Harden from the University of Oxford in the United Kingdom presented data on 11 patients treated with polyclonally-expanded $T_{reg}$ which were infused shortly after kidney transplantation.[26] No adverse events related to the infusion were observed and patients showed excellent kidney graft function. This study demonstrated elegantly that $T_{reg}$ therapy is feasible and may be efficacious. However, the transient risk of generalized immunosuppression by these nonspecific $T_{reg}$ cannot be ignored, since only a low percentage of the polyclonally-expanded $T_{reg}$ are alloantigen specific. For this reason, and because animal studies have shown that antigen-specific $T_{reg}$ are more potent, it may be preferable to treat patients with antigen-specific $T_{reg}$. Megan Levings from the University of British Colombia in Canada spoke about how the hurdles of expanding antigen-specific $T_{reg}$ can be overcome. First, alloantigen-specific $T_{reg}$ can be expanded, but this is a slow process and requires access to donor antigen. Second, a transgenic T cell receptor (TCR) approach can be followed although this is quite time consuming, Third, the chimeric antigen receptor (CAR) approach which has been developed in the field of oncology can be utilized. In her presentation, Levings demonstrated that these CAR $T_{reg}$ are potent suppressors of alloreactivity. It is hypothesized that patients who are HLA-A2 negative and receive a HAA-A2 positive kidney will receive $T_{reg}$ which also recognize HLA A2 and control the anti-donor response to protect the transplanted organ.[27] In a xenograft versus host model, these CAR $T_{reg}$ effectively protected the animals from disease development. Thus, CAR technology means it is now possible to generate

potent, functional, and stable alloantigen-specific human $T_{reg}$ that will markedly enhance their therapeutic potential in transplantation.[27]

New mechanistic data have been presented by Bézie from INSERM at Nantes in France and by Bromberg from the University of Maryland in the United States. These teams have explored the role of cytokines in $T_{reg}$ function and the impact of lymph node basement membrane proteins on $T_{reg}$ proliferation and polarization. Bézie observed that IL-34, a cytokine which signals via the M-CSF receptor and promotes monocyte survival, also promotes $T_{reg}$ function.[28,29] In their studies, polyclonally-expanded $T_{reg}$ were more potent suppressors when grown in the presence of IL-34.[29] Bromberg presented intriguing data indicating that that proteins called laminins, which contribute to the structure of extracellular matrix, also influence the action of T cells. In a pro-$T_{reg}$ environment, laminin 511 inhibited $T_{reg}$ induction, an effect for which alpha 6 integrin is essential, while in an IL-17 milieu laminin 511 favored Th17 induction via α dystroglycan. These studies show that lymph node laminins act as molecular switches for tolerance and immunity by directly influencing T cell proliferation and polarization.[30]

In addition to treating patients with cells to control the anti-donor response, or even induce tolerance, there is also great interest in tools that can identify whether patients have developed tolerance or have an immunologic quiescent profile so that immunosuppression can be tapered accordingly. A group working with Megan Sykes at the Columbia University Medical Center in the United Stated has used the classic mixed lymphocyte reaction, combined with T cell receptor sequencing, to measure the anti-donor T cell receptor repertoire before transplantation. This is

then compared to the repertoire after transplantation to identify and track alloreactive clones. Their first data showed that tolerant kidney transplant patients show significant deletion of T cell clones.[31] Future studies are needed to confirm if this assay can indeed be used to detect patients who have become tolerant.

*B cells and the humoral anti-donor response*

In an early morning session on *'B cells and antibodies'*, Dr Di Yu from Monash University in Australia presented data on the mechanisms and cells required to produce high-affinity antibodies in a talk entitled 'W*hat are the key signals for B cell activation?*. Yu explained that follicular T helper (Tfh) cells are essential for the anti-donor B cell response. These Tfh cells reside in the germinal centers of lymph nodes and spleen, where they contribute to antigen-activated B cell activity via co-stimulation signals such as CD40L, ICOS and PD-1, and by production of IL-21.[32] Studies by Kitty de Leur and colleagues have demonstrated that lymphoid follicles and Tfh cells are present in the transplanted kidneys of immunosuppressed patients.[33,34]

In chronic antibody-mediated rejection, one of the main causes of late organ failure, serum donor specific antibodies (DSA) are an important and well-established diagnostic biomarker. At the meeting, several talks described the latest developments for improving the prognostic value of assays which detect and quantify DSA and anti-HLA antibodies, particularly in patients with a poor prognosis due to the presence of complement-fixing DSA.[35] Lionel Rostaing from Grenoble in France presented data showing that complement activation might depend on the concentration of DSA.[36] Dr Cai from the Terasaki Foundation laboratory in Los Angeles discussed how C1q-

fixing antibodies against denatured HLA and MICA antigens are associated with antibody-mediated rejection after kidney transplantation.[37]

Anti-donor antibodies are mostly of the IgG subtype which binds to both HLA and non-HLA antigens expressed by the grafted organ, and are responsible for the pathology which reduces graft survival. IgG antibodies activate complement and can also engage with the surface $F_c$ gamma receptors that are expressed on macrophages, dendritic cells, neutrophils and natural killer (NK) cells. Dr. Menna Clatworthy from the University of Cambridge in the United Kingdom spoke about the available immunosuppressive agents which specifically target B cells and antibody-producing cells.[38] She proposed a concept whereby nonsensitized patients should receive treatment that prevents naïve B cell activation, while in patients who have developed *de novo* antibodies or have an acute B cell-mediated rejection episode the plasma cell germinal center reaction should be prevented. In sensitized DSA-positive patients, memory B cells and long-lived plasma cells should be targeted. Thus, instead of a 'one-size-fits-all' strategy for global B cell depletion in patients with antibody-mediated rejection, more targeted therapy should be applied to achieve optimal efficacy with minimal safety concerns. Unfortunately, few studies reporting the outcomes of randomized controlled trials targeting B cells and/or plasma cells were presented at the meeting. Such studies should be initiated to establish their efficacy and safety profile in transplant patients. A first indication may come from a Phase 2 safety study targeting the B cell activating factor (BAFF) molecule belimumab in kidney transplant patients (www.clinicaltrials.gov/ NCT 1536379).

**How will transplantation change over the next five years?**

At the end of the 5-day meeting is was clear that there are no simple answers to improving long-term graft and patient outcomes after solid organ transplantation. This report touches on only a few of the many highlights of the Congress. No less important are recent insights into the role of microbiota on the immune system, monocytes and dendritic cells, NK cells and trained immunity, memory T cells, T cell exhaustion, co-stimulation and co-inhibition, and biomarkers such as cell free (cf) donor DNA.

Over the years we have learned that complications affecting outcomes after transplantation have multiple causes, involving immunological, pro-fibrotic and inflammatory processes as well as the consequences of lifelong treatment with toxic immunosuppressive drugs. The transplant community can collaborate, sharing protocols, data and patient materials to speed up the discovery process. Within five years, it is quite feasible that we will 1) work with standardized reagents, equipment and techniques in the laboratory to increase the quality of results; 2) have a set of reliable biomarkers to predict late graft failure; 3) treat type I diabetic patients with embryonic stem cells(ES) and or iPS (induced pluripotent stem cells) generated ß-cells; 4) apply cell therapy with $T_{reg}$ on a much larger scale and 5) have developed anti-B cell therapies to prevent and treat antibody-mediated rejection implemented in the clinic. These advances represent key steps to further improving long-term graft and patient outcomes after transplantation.

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