R O T R F

Roche Organ Transplantation Research Foundation

G R A N T A W A R D S

CYCLE 1 – awards in April 1999

1. Introduction

The ROTRF completed its first competition in March 1999, thanks to its outstanding panel of Scientific Advisors. The unique challenges posed by a worldwide competition to grant a small number of awards were addressed via a fully electronic submission process for letters of intent, followed by a full paper grant application. This system allowed adjudication by a busy panel of Scientific Advisory Committee (SAC) members, without requiring face-to-face meetings. The Board of Trustees made the final decisions based upon the recommendations of the SAC members.

Both the Board of Trustees and the Committee have been very pleased with the quality of the applicants and their submissions, and look forward to the coming cycle.

The ROTRF welcomes any feedback or creative suggestions to assist the Foundation in accomplishing its stated mission in advancing the field of solid organ transplantation.

On behalf of the Board of Trustees

Phil Halloran

FACTS AND FIGURES

CYCLE 1 – OCTOBER 1998

A total of 170 applications for funding were reviewed by the Scientific Advisory Committee of the Roche Organ Transplantation Research Foundation. The ROTRF, which was launched at the International Society for Organ Transplantation meeting in Montreal in July 1998, received applications from a wide range of countries (please see table).

Of these applications, 15 were invited to submit full paper submissions. The largest number of applications (73) was received from the USA, with Canada second at 15. The greatest number of applications from a European country was 15 (Germany and England).

In this first cycle, 3 million Swiss Francs were disbursed for grants in total.

Country	No.	Country	No.	Country	No.
Argentina	1	Australia	11	Austria	2
Belgium	2	Canada	15	Chile	1
England	15	Finland	2	France	9
Germany	15	Hungary	1	Israel	3
Italy	5	Netherlands	5	Pakistan	1
Romania	1	Scotland	1	Switzerland	4
South Africa	1	Spain	1	Sweden	1
USA	73				

Principle Investigator
Prof. Anthony J.F. d'Apice
Dr. Peter J. Cowan – Co-applicant
Dr. Trixie A. Shinkel – Associate

St. Vincent's Hospital Melbourne – Australia

Project:

Prevention of rejection of organ transplants between species by antioxidant genes

Transplantation of organs between different species (e.g. pig to human) is a possible solution to the chronic shortage of human organs for transplantation. However, this is currently not possible because the human immune system recognizes the pig organ as foreign and mounts a rapid and vigorous rejection response. This rejection response can be divided into two stages: hyperacute rejection and acute vascular rejection. The first stage occurs within minutes to hours by binding of human antibodies to the surface of the pig organ and subsequent activation of the human immune system. It is now generally accepted that this process can be overcome by genetic modification of the donor organ. The next phase of rejection occurs over a period of days to weeks and is a complex process involving multiple factors. One damaging factor believed to be involved in this process is oxidative stress. Under normal conditions, a balance between oxidants and antioxidant mechanisms is maintained due to the presence of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. If this balance is disrupted to favour the overproduction of oxidants, as occurs during transplantation of organs, the antioxidant enzymes will no longer be able to cope with the removal of excess oxidants and thus loss of the donor organ will occur. The hypothesis of this study is that increasing the expression of antioxidant enzymes in the donor animal using current genetic technology will protect the organ from injury caused by oxidants and thus prolong survival. Initial studies will be carried out to determine the best combination animals. Animals expressing this combination of antioxidant enzymes will be examined to determine whether their organs are protected from transplantation-associated injury.

Principle Investigator
Dr. Jorge A. Bezerra
Dr. David Witte

University of Cincinnati, Cincinnati – USA

Project:

Role of proteins to improve outcome of liver cell transplantation

Hepatocyte transplantation is an emerging treatment modality for patients with liver failure of hereditary metabolic diseases. In this technique, hepatocytes (the main liver cell) are injected into the vascular bed of patients with liver disease. Following injection, hepatocytes migrate to the liver, become part of the native liver cells, and function normally. Despite the pioneering use in patients with liver failure as a bridge to liver transplantation, the use of hepatocyte transplantation remains limited because only a small number of cells successfully populate the recipient liver. Although little is known about the factors which control the outcome of hepatocyte transplantation, recent studies in genetically modified mice demonstrated that in the appropriate biological setting transplanted cells can repopulate almost the entire liver. These mice produced an elevated amount of urokinase, a protein involved in the reorganisation of the structural scaffold of the liver and other organs. Data from our laboratory demonstrated that urokinase creates a microenvironment in the liver which is conducive to expansion of transplanted hepatocytes. In this grant, we propose to use mice with genetic modifications of urokinase and of other functionally related proteins in transplantation experiments to define their role in the outcome of hepatocyte transplantation. Our strategy will be to transplant hepatocytes from these mice into control mice. We will then use gene markers and morphological analysis to identify and count the number of transplanted hepatocytes in the recipient livers. We expect that the production of these proteins by donor hepatocytes will lead to improved outcome following transplantation. These experiments are likely to identify key molecules that can be used in the clinical setting to optimise the use of this technique in the support of patients awaiting liver transplantation and in the treatment of patients with liver disease.

Principle Investigator
Dr. Jeffery A. Bluestone
Dr. Matthew D. Griffin, Co-Applicant

University of Chicago, Chicago – USA

Project:
Blockade of organ graft rejection by CTLA-4 ligation

Transplantation has become an established treatment for illness involving failure of function of one or more organ systems. Over the past three decades, considerable improvements have been made in short-term outcomes for all commonly transplanted organs. However, progress in overcoming the long-term limitations of transplantation has not been as successful. Chronic graft dysfunction, increased risk of infection and malignancy, and other immunosuppression-related effects such as increased vascular disease, hypertension and osteoporosis remain major limitations to the quality of life and survival of organ recipients.

The T cell response has evolved to distinguish between self and foreign tissue. Thus, exposure of the immune system to a foreign organ triggers a rejection episode mediated by antigen-specific T lymphocytes. The goal of our project is to develop new therapeutics to block the activation of the T cells that destroy the foreign organ. We have discovered that, under certain circumstances, T cell responses to foreign tissues can be modified such that they fail to respond and to not reject the transplant tissues. This phenomenon, termed tolerance, takes advantage of natural processes normally involved in regulating self-reactivity that prevents autoimmune disease in most people. Thus, treatment protocols aimed at promoting donor-specific tolerance as a replacement for life-long immunosuppression holds great promise for improving the current status of organ transplantation.

Over the past several years, we have identified a molecule on the surface of activated T cells, CTLA-4, that can shut down early events in the activation of T cells. CTLA-4 regulated transplant rejection as cardiac allograft rejection is accelerated in a rodent model following CTLA-4 blockade. This has led us to hypothesise that productive engagement of CTLA-4 during graft recognition by T cells may result in reduced immune responses to graft antigens and favour tolerance. We propose to apply gene therapy techniques to introduce innovative strategies to turn off T cells using a special form of an anti-CTLA-4 antibody that is expressed on the surface of the transplanted tissue that exploit the negative signalling properties of CTLA-4. We hope that these novel approaches will prevent graft rejection and induce tolerance in solid organ transplant recipients.

Principle Investigator
Prof. Alfred L.M. Bothwell

Yale University, School of Medicine – New Haven, CT – USA

Project:
Characterization of molecules important for preventing rejection of transplanted pig organs

The only successful method of treatment of certain diseases especially of the kidney and heart is by organ transplantation. In addition, while many patients undergoing dialysis do survive for a long period of time there is significant mortality. An enormous improvement in lifestyle can be achieved by transplantation. Unfortunately, the supply of donor human organs can not even begin to meet the current needs of patients and one would anticipate that it never will. Therefore, development of alternative strategies that would meet this need would be of enormous benefit to society.

At the current time by far the most favoured source for donor organs is the pig. Indeed, there is not any other contender given the potential advantages of supply and ability to create transgenic animals. In the last few years, the biologic basis for the rapid rejection of potential porcine organs has been defined. Natural antibodies in humans react with molecules on porcine cells that result in destructive action by the complement system. Strategies have now been devised using complement inhibitors that appear to overcome this major short-term biologic barrier.

The long term survival of porcine grafts will require a thorough understanding of the cellular immune responses that occur in vivo. The primary regulators of the human anti-porcine response that must be characterized are distinct populations of T lymphocytes. T cells recognize proteins on porcine cells that promote direct binding as well as signal the T cells that they are foreign. The focus of this proposal is the identification and characterization of these porcine cell proteins that affect the immune response of human T cells. This information is required for development of either small molecule inhibitors, transgenic porcine organs of humanized antibody reagents that will enhance the survival of xenografts.

Principle Investigator
Dr. Julie Dechanet
Dr. Jean-Francois Moreau, Co-Applicant
Dr. Pierre Merville, Co-Applicant
Dr. Vincent Pitard, Associate
Dr. Xavier Lafarge, Associate

Bordeaux University 2, Bordeaux, Cedex France

Project:

Analysis of the mechanisms used by the immune system to fight against the Cytomegalovirus

The cytomegalovirus (CMV) is widely encountered in human and usually not dangerous for healthy individuals. In contrast, primo-infection or reactivation of this virus in immune-suppressed individuals such transplanted or AIDS patients, induce a severe disease. The aim of this project is to understand the mechanisms evolved by the human immune system to fight against this virus, in order to find new therapeutical strategies to prevent this disease in immunosuppressed patients. For the first time, we recently observed that particular cells of the blood (gamma-delta T lymphocytes) could be involved in the response of the immune system directed against CMV in renal transplanted patients. We now want to investigate the specific interactions between these cells and the CMV to understand how they can help to eliminate this virus.

The Board of Trustees decided to honour Prof. Halloran with an honorary founder's award in recognition of his dedication to the Foundation of the ROTRF and his excellence in scientific research.
Prof. Halloran's letter of intent and full paper submission were reviewed with the same evaluation process by the SAC as all other grants and were both highly rated by the SAC.

Principle Investigator
Prof. Philip Halloran
Dr. Calvin Harley
Dr. Walter Funk
Dr. Nam Kim
Dr. Kim Solez
Dr. Ron Moore
Dr. Gerald Todd

University of Alberta, Edmonton, Alberta – Canada

Project:
Exploring How Aging Mechanisms Affect Transplants
Background: Kidneys from older donors are more prone to fail but the mechanism for this is not known. This is concerning since the organ shortage forces us to use older donors. In addition some evidence suggests that aging may be accelerated in transplants because of the abnormal stresses of transplantation. Aging changes reflect the presence of "clocks" in the tissue, one of which may be the ends of the chromosomes, the "telomeres". Telomeres shorten with age until critically short telomeres cause cells to die. We recently showed that telomere shortening can be detected in old kidneys.

Hypothesis: 1. Old kidneys will show changes like "senescent" cells in culture. 2. Transplantation accelerates the aging process in kidneys.

Proposal: To examine telomere shortening and patterns of senescence gene expression in normal and diseased kidneys and in kidney transplants; to determine whether transplantation accelerates telomere shortening.

A. Reading the "clocks" in kidneys: We will establish new methods to measure telomeres. We will see if these changes are related to kidney function, how tissues change as the clocks advance, and whether kidney disease changes the clocks.

B. Senescence and stress changes in transplanted kidneys at the time of transplantation in relationship to the clinical course: We will determine whether telomere length in kidney at donation predicts transplant function and survival. We will determine whether telomere shortening and senescence is increased in kidneys developing chronic allograft nephropathy (CAN).

C. Mouse M Spretus has telomeres like humans and can be used to test whether injury advances the aging clocks.

Significance: Understanding aging and "senescence" may permit us to predict transplant survival and may guide treatment to avoid additional injury in kidneys at high risk of CAN. We may better decide which old organs can be used safely.

Principle Investigator
Dr. Jon S. Odorico
Dr. Nadya Lumelsky, Associate

University of Wisconsin, Madison, WI – USA

Project:
Derivation of Insulin Producing Cells from Embryonic Stem Cell Lines

Transplantation of the whole pancreas and isolated pancreatic islets has greatly improved the treatment of diabetes mellitus in recent years. However, transplantation of organs from cadaver donors cannot be offered to all diabetics because of the severely limited supply of suitable donors, and because of the often fatal immunological complications associated with such organ transplantation. Stem cell therapy is beginning to emerge now as a potential alternative strategy for the treatment of many human diseases. The aim of stem cell therapy is to replace, repair or enhance the physiological function of damaged tissue or organs by providing the recipient with the cells that can be engineered outside the body to serve this physiological function. Embryonic stem (ES) cells represent a very promising source for stem cell therapy because they are immortal, can be grown in large quantities, and can be genetically engineered outside the body to escape immune attack by the recipient. Moreover, ES cells are capable of giving rise to serveral different functional cell types. For instance, cardiac muscle cells, nerve cells, and red blood cells have been grown from ES cells in tissue culture. We have recently found that ES cells can also be induced to produce some of the pancreatic hormones, but this hormone production is still relatively inefficient. In the proposed work, we are undertaking the task of transforming ES cells into functional insulin-producing cells by further enhancing their hormone production while expanding the number of hormone-producing cells. Achieving this goal will have important therapeutic benefits for treating diabetes.

Principle Investigator
Dr. Miguel P. Soares
Prof. Fritz Bach, Co-Applicant

Harvard Medical School, Boston, MA – USA

Project:
Mechanisms of action of protective genes that promote organ graft survival

Organ allotransplantation is among the important medical advances of this century. However, the success of this approach has created a new problem: the shortage of donor organs available for this procedure. Based on this consideration, xenotransplantation of pig organs into humans is considered as a potential solution. The major obstacle to the clinical applications of xenotransplantation is that porcine organs are always rejected when transplanted into primates. In our center we use as an experimental model to study the mechanisms involved in xenograft rejection the transplantation of mouse hearts into rats. We have recently developed an immunosupressive treatment that allows such xenografts to survive indefinitely. This grant is based on the new finding, by our group, that mouse to rat cardiac xenografts that survive indefinitely under this immunosuppressive protocol protect themselves against rejection. The mechanism underlining this phenomenon relies on the expression, by endothelial cell (EC) lining the surface of xenograft blood vessels, of a series of "protective genes". These genes protect EC from dying and in addition inhibit the expression of pro-inflammatory genes that presumably contribute to xenograft rejection. We will study one of such protective genes, heme-oxygenase-1 (HO-1), the expression

of which we have shown to be essential to insure the survival of mouse hearts transplanted into rats. One of the main goals of this proposal is to analyze the cellular/molecular mechanisms by which expression of HO-1 in EC protects mouse to rat cardiac xenograft from being rejected. The other main goal is to analyze whether the transgenic overexpression of HO-1 in the xenograft endothelium would prevent the rejection of mouse to rat cardiac xenografts, under conditions leading to the rejection of non-transgenic grafts. We believe that the results gained in this application will provide innovative new approaches to overcome xenograft rejection in a clinically relevant situation such as in pig organs transplanted into primates.

Principle Investigator
Prof. Angus Thomson
Dr. Adrian Morelli, Co-Applicant, Dr. Petra O'Conell, Associate
Dr. Mohamed H. Sayegh, Consultant. Dr. Charles Maliszewski, Consultant

University of Pittsburgh, Pittsburgh, PA – USA

Project:
Studies of a novel cellular regulator of host response to organ transplants
The host response to organ transplants is initiated and controlled by specialised white blood cells termed dendritic cells (DC) that are present in the graft and in the blood and lymph glands of the host. Elucidation of the role(s) of these cells in presenting foreign (antigenic) material and in regulating the immune response is crucial to further understanding the mechanisms that led either to graft rejection or acceptance. Although DC have been studied by a small number of research groups for the past 25 years, there has been a recent dramatic increase in our understanding of the properties of these cells based on technologic advances that permit their enhanced production and characterisation. This new knowledge includes recognition that DC are much more diverse in character and function than previously recognised. Amongst these recent discoveries is the identification of a major novel population of DC termed lymphoid DC (LDC). Based on limited studies to date it has been suggested that while DC that have been studied for many years ("myeloid" DC or "MDC") are primarily instigators of immune responses, the newly identified LDC may be more important in immune regulation, including the potential to promote "tolerance" (permanent donor-specific unresponsiveness) to "foreign" material (antigens), as represented by an organ transplant. This research project will more fully characterise LDC in terms of their functional interactions with T lymphocytes from unrelated ("foreign") individuals, both using cell culture techniques and following the injection of LDC into experimental animals (mice) including recipients of organ (hearts) grafts. It is expected that these LDC will demonstrate the potential to modulate host responses to organ transplants and that the results will provide a basis for further evaluation of their therapeutic potential.

Principle Investigator
Dr. Hans-Dieter Volk
Dr. Birgit Sawitzki, Co-Applicant
Dr. Gerald Grütz, Associate
Dr. Petra Reinke, Associate

Institut for Medizinische Immunologie – Charité, Berlin – Germany

Project:

Acceptance of foreign organ transplants – How can we outfox the immune system?

Today organ transplantation is a well-established method for replacement of irreversible deteriorated organs such as kidney, heart, liver, lung etc. For this approach we are using organs from living or cadaver donors (allografts). Because the allografts carry foreign proteins, our immune system will be activated and rejects the allograft if we do not suppress it by drugs. These immunosupressive drugs the graft recipient has to take as long as the graft is working – as result of the chronic immunosupression several side effects can be observed. The dream of all transplant physicians is to develop strategies to outfox the immune system – to induce transplantation tolerance. In animal models this is already realism. In order to transfer the promising data from the animals to the human situation, we have to learn more about the mechanisms. The aim of this project is to give substantial information for our understanding of 'transplantation tolerance' by using modern gene technique approaches.

To apply:

Internet: **http://www.ROTRF.org**

E-mail: admin@ROTRF.org
Tel.: +41 41 377 53 35
Fax: +41 41 377 53 34
Mail: ROTRF, Postfach 222
6045 Meggen, Switzerland

4.99, X-Ray