R O T R F

Roche Organ Transplantation Research Foundation

G R A N T A W A R D S

CYCLE II – awards in October 1999

Table of Contents

1. Preface

Another cycle of reviewing research proposals has gone by for the Roche Organ Transplantation Research Foundation (ROTRF).

We are very satisfied with the excellent review process of the ROTRF, which is now well established. Due to the electronic submission of a short letter of intent – a descriptive abstract of the submitted research proposal – applications were efficiently processed and rated. The top-ranked applicants were subsequently invited to submit a full paper application, with an approximately 50% chance of funding. Therefore, the ROTRF's application and review process has proven itself to satisfactorily balance a good probability of success for applicants and the selection of high-quality proposals for support by the Foundation. Thanks to the excellent reviews by the Scientific Advisory Committee, highly qualified research projects have been selected for funding, and are expanding the repertoire of ROTRF-supported grants.

The mission of the ROTRF is to support research relevant to progress in solid organ transplantation. We have emphasized the need for innovation and novelty, and for thinking "outside the box." In keeping with this principle, the ROTRF seeks to foster and nurture this creativity. We want to attract more researchers outside of the direct transplantation area in the hope of encouraging those with innovative scientific ideas and approaches to apply themselves to transplant-related problems. We also seek to encourage more applications from female scientists. To achieve these goals in the future, we plan to communicate the Foundation's mission and potential to a broader scientific community.

Although the projects represent early stages of the process by which discovery influences therapy, they represent important first steps in new areas that will lead to new understanding, new clinical applications, and improved outcomes. Moreover, the analogy of kissing frogs to find a prince comes to mind: ideas must

be tried, and many will be proven to be incorrect. But even proving that a particular frog is not a prince is progress. Moreover, it is usually a long way from the concept to the clinical application, even when the idea is correct. The funded research projects will contribute to knowledge about many aspects of the clinical and scientific adventure of transplantation, e.g. the mechanisms of rejection and tolerance, the mechanisms of long-term organ deterioration, the consequences of tissue injury, and the opportunities to intervene in these processes.

All those who have been involved in the successful launch of the ROTRF deserve the Foundation's gratitude. We welcome any feedback and support to assist the Foundation in accomplishing its mission.

On behalf of the Board of Trustees

Phil Halloran

2. Facts and Figures

CYCLE II – Submission April 1999

In the second cycle of Grant Awards, the Roche Organ Transplantation Research Foundation (ROTRF) received 149 applications from 25 different countries (see table below). The greatest number of research proposals was submitted from the USA (38%). Most of the European proposals came from Germany, England and France, representing together 31% of the submitted applications. Altogether, women represented 17% of the applicants.

Country	No.	Country	No.	Country	No.
Australia	5	Hungary	1	Portugal	1
Austria	1	India	1	Russia	1
Belgium	3	Ireland	2	Singapore	1
Canada	6	Israel	2	Sweden	1
England	16	Italy	6	Switzerland	3
Finland	1	Korea (South)	1	USA	58
France	12	Lithuania	1	Yugoslavia	1
Georgia	1	Netherlands	6		
Germany	17	New Zealand	1		

The Scientific Advisory Committee of the ROTRF reviewed all 149 applications that had been submitted electronically on the ROTRF's homepage (www.ROTRF.org). The research proposals were ranked according to scientific excellence, originality and relevance to solid organ transplantation. The top 16 applications were invited to submit a full paper application and underwent subsequently a second thorough review by the Scientific Advisory Committee and the Board of Trustees.

The ROTRF Grants were finally awarded to 9 applicants, 4 from the USA, 3 from Europe and 2 from Canada. They all presented excellent and very promising research projects (see abstracts on the following pages). Their research interests focus mainly on induction of tolerance, the development of new agents and relevant immune recognition, regulation and effector mechanisms.

In this second cycle of ROTRF Grant Awards, 1.9 million Swiss francs were disbursed in total.

Further information about the ROTRF, its charter, statutes and general organization can be found on the ROTRF homepage **(www.ROTRF.org).** It contains all necessary guidance and instructions for applying to the ROTRF for funding. Any application for funding starts with the submission of a letter of intent directly on the ROTRF homepage.

3. Grant Awards in Cycle II

Principle Investigator
Prof. Jonathan S. Bromberg

Mt. Sinai School of Medicine, New York – USA

Project:
Gene Therapy to Prolong Graft Survival

While organ transplantation is generally successful, there are still a number of significant problems that affect all transplant patients. Most transplanted organs are eventually rejected after a period of several years, and most patients have to take multiple immunosuppressive medications for the rest of their lives, or at least while the transplant is functioning. These medications are expensive, cause a number of significant side effects and do not ensure indefinite survival of the transplanted organ. Thus, there is a critical need to understand more completely the immune system, so that new techniques and new drugs can be developed that will ensure better organ survival, while decreasing the side effects of the treatments.

This research application will investigate in particular the effectiveness of gene transfer and gene therapy techniques in transplantation. Gene therapy has the potential to deliver immunosuppressive drugs directly to the organ, thereby avoiding drug side effects to the other organs of the patient. Gene therapy also has the potential to deliver novel drugs that have never been used before in transplantation. This research application proposes to use gene therapy to deliver a new class of drugs, called chemokine inhibitors, to organ transplants, and determine if they promote the survival of the transplant.

Principle Investigator
Dr. Régis Josien
Dr. Cristina Cuturi, Co-Applicant
Prof. Jean-Paul Soulillou, Co-Applicant
Cécile Voisine, Research Assistant
Dr. Patrick Mathieu, Research Assistant
Dr. Yongwon Choi, Consultant

ITERT/INSERM Unit 437, Nantes – France

Project:

The Role of TRANCE during Allograft Rejection and Tolerance

The understanding of immune mechanisms leading to allograft rejection and allograft tolerance is an important step toward the identification of molecular targets of current and new immunosuppressive drugs. Recent results have shown that the molecule CD40 ligand (CD40L) which is expressed on activated CD4+ T lymphocytes and its counter-receptor CD40 which is expressed on antigen presenting cells (dendritic cells, macrophages, B lymphocytes) represented a major pathway for T cell activation both *in vitro* and *in vivo.* Monoclonal antibodies that antagonize CD40L have been shown to delay allograft rejection in rodent and monkey models and will probably be used in the future in human organ transplantation. Recently, a new member of the tumor necrosis factor (TNF) family, called TRANCE (TNF-related activation induced cytokine), has been described. This molecule shares functional properties with CD40L but appears to be more specialized in the interaction between T cells and dendritic cells, two cell subsets which play a pivotal role in immune responses to allografts. *In vivo* studies suggest that, in the absence of the CD40L/CD40 pathway, the immune system can use the TRANCE-TRANCE-receptor pathway to activate T helper cells. In this proposal, we will analyze the role of TRANCE/TRANCE-receptor interactions during immune responses leading to allograft rejection and allograft tolerance using a rat model of heart allograft. The expression of TRANCE and its receptor will be assessed in allografts and lymphoid organs. TRANCE-blocking bioreagents will be used *in vivo* and will allow us to determine the role of this pathway in experimental allograft rejection and tolerance. Based on these studies and their results, we will design new experiments to test the effects of TRANCE-blocking bioreagents in a monkey model of renal allograft.

Principle Investigator
Dr. Lina Lu
Dr. Andrew Bonham, Co-Applicant
Dr. Nick Giannoukakis, Associate

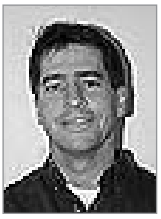
University of Pittsburgh, Pittsburgh – USA

Project:
Prevention of Heart Allograft Rejection by Genetically Modified Immunoregulatory Cells

At present, therapy of organ transplant rejection is dependent on immunosuppressive drugs. These agents, which may need to be taken for the lifetime of the patient, depress the whole immune system, and often result in increased risk of infections, and in some cases, cancer. The host response to organ transplantation is initiated and controlled by a specialized population of white blood cells, termed dendritic cells (DC). We have demonstrated recently that the administration of donor immature DC can be used to regulate recipient immune responses, and to inhibit the specific response against graft antigens without impairing the whole immune system. Although this approach offers several advantages, a drawback is that immature donor DC may become mature at some stage after their injection, and elicit harmful immune responses to the graft. Therefore, arresting of DC maturation is extremely important for the further development of a DC-based antirejection therapy. One of the important molecules that regulates DC maturation and function is nuclear transcription factor κB (NF-κB). Our preliminary studies have shown that blockade of this molecule by NF-κB specific oligodeoxyribonucleotide (ODN) decoys significantly inhibits DC maturation. Administration of DC genetically modified with NF-κB ODN (NF-κB ODN DC) prior to heart transplantation significantly prolongs allograft survival in mice. DC treatment with NF-κB ODN is safe, efficient, and inexpensive.

In this proposal, we will determine the optimal conditions for the generation of NF-κB ODN DC, and ascertain the most appropriate regimen of NF-κB ODN DC-based therapy for prevention of organ allograft rejection in a mouse-vascularized heart transplantation model. Positive results from this proposal will provide a basis for a trial of a genetically modified DC therapy in patients receiving organ transplants.

Principle Investigator
Dr. Sean O'Herrin
Dr. Kenneth A. Newell, Collaborator
Dr. Jeffrey A. Bluestone, Collaborator



University of Chicago, Chicago – USA

Project:
Utilization of Immune System Molecules to Prolong Survival of Transplants

Illnesses such as kidney failure, diabetes and liver disease have a devastating effect on public health. For such diseases (and for a range of other diseases), the accepted treatment is organ transplantation.

Over the past decade, there has been remarkable progress in organ transplantation, and it is now the treatment of choice for the failure of organs such as the pancreas or kidney. In many cases, however, the benefit of transplantation may be short-lived. In fact, nearly one-third of all patients on waiting lists for donor organs have had their previous transplant fail. The failure of a transplanted organ is almost always due to a process called rejection.

The rejection of a tissue or organ transplant occurs because our white blood cells (or T cells) in our immune system are trained to protect us from foreign invaders. A special molecule found on virtually every cell, known as MHC, tells the T cell if foreign material from say, a viral or microbial invasion is present. Transplanted tissues, unless they are procured from an identical twin, are recognized as foreign, and are consequently attacked by "activated" T cells that have been targeted to the transplant, eventually damaging or destroying the graft.

It may now be possible to prevent this process by destroying or inactivating these activated T cells. However, care must be taken not to destroy the other "good" T cells that maintain protection against bacteria, viruses, etc. We have designed a series of custom MHC molecules that mimic transplanted tissues. The activated T cells, believing these molecules to be the transplanted tissue cells, lock on the MHC. Since these MHC molecules are not on a cell surface, but essentially out of context, they trick the T cells into a state of inactivation. Our "pepMHC" combinations will be tested in animals receiving organ transplants in order to test their effectiveness in preventing transplant rejection.

Principle Investigator
Dr. Risto Renkonen
Dr. John B. Lowe, Co-Applicant

The Haartman Institute, University of Helsinki,
Helsinki – Finland

Project:
Prevention of Transplant Rejections by Blocking White Cell Traffic into Grafts

Acute heart allograft rejection is characterized by heavy infiltration of white cells, namely lymphocytes. To infiltrate the graft and thus promote rejection, the lymphocytes extravasate from blood through the vascular endothelial layer into the graft parenchyma. Extravasation of lymphocytes is initiated by an interaction of members of the selectin family and their oligosaccharide-containing ligands. L-selectin is expressed on leukocyte surfaces and recognizes its endothelial protein counterreceptors, provided that they are decorated with properly a 1,3-fucosylated glycans, the prototype decoration being sialyl Lewis x (sLex) for L-selectin. Importantly, endothelium under normal conditions does not express proper glycoforms of L-selectin ligands. However, proinflammatory stimuli in our *in vitro,* animal and most recently human heart transplantation patients studies have shown that endothelium can be induced to express these glycans *de novo* and to promote leukocyte extravasation. A novel approach to increase immunosuppressive efficacy, without targeting to activation and proliferation of T cells, would be to inhibit the carbohydrate-dependent entry of lymphocytes into the graft.

Our work with experimental animals has demonstrated that free sLex-containing glycans can prevent short-term selectin-dependent inflammation, for example, after reperfusion injury. With rat heart and kidney allograft models, we have shown a strong induction of sLex-decorated L-selectin ligands on only microvascular endothelium occurring concomitantly with the accumulation of lymphocytes into the grafts. The proposal focuses on the use of novel selectin-ligand knockout mice in heart transplantation experiments. Here we address the role of selectin ligands to the initiation of allograft rejection.

Principle Investigator
Dr. David H. Sachs
Dr. Kazuhiko Yamada, Co-Applicant

Massachusetts General Hospital, Boston – USA

Project:
Thymic Transplantation to Achieve Tolerance

One of the major limitations to the field of transplantation today is the insufficient supply of donor organs. The current proposal is directed toward the eventual goal of making it possible to transplant organs from other species (a procedure called "xenotransplantation") in order to overcome this limitation.

Our plans involve transplantation of the thymus, which is the lymphoid tissue of greatest importance in the immune response to transplantation. The thymus is the site where new immune cells, which are continuously formed throughout life, are selected not to react against "self," which would otherwise cause auto-immune disease. The result of this selection is called immunologic tolerance. Because the immune response across species is extremely strong, we hope to induce tolerance to xenotransplants, rather than relying on very high doses of immunosuppressive drugs, which would have serious side effects.

We will transplant the thymus of donor animals to potential recipients, in an attempt to induce tolerance to other tissues and organs from the same donor. It is known that transplants of tissues like the thymus are more readily accepted if they have their own blood supply than if they must establish new blood vessels from the recipient in order to survive. For this reason, we will transplant composite organs into which the donor animal's own thymic tissue has been implanted several months prior to the organ transplant. This procedure gives the thymus tissue sufficient time to heal in, so that when organ transplantation is performed, the thymus does not need additional time to become vascularized.

We have already shown in miniature swine that this process is successful across limited transplant barriers within a species. We will now try to apply it to highly mismatched transplants within this species, as well as from swine to baboons, a species barrier that is very relevant for eventual clinical xenotransplantation.

Principle Investigator
Prof. Kathryn Wood

University of Oxford, Oxford – United Kingdom

Project:

Identity of the Cells Responsible for Transplant Survival

The survival of a transplant is currently dependent on the continued administration of powerful immunosuppressive drugs. Although effective in the short term, nonspecific immunosuppressive drugs appear unable to switch off the immune response to the donor completely. In other words, they are unable to induce unresponsiveness or tolerance to the transplant. The induction of tolerance to the organ donor is one of the important aims for the future development of clinical transplantation. Switching off all or even part of the immune response to a transplant would diminish the requirement for long-term immunosuppressive drug therapy thereby reducing the risks of infection and cancer after transplantation.

There are a number of new and exciting approaches for manipulating the immune response after transplantation. Some of these involve the use of biological molecules that in addition to preventing rejection may also facilitate the development of tolerance. At the moment, there are no reliable ways of monitoring whether tolerance to the organ donor has developed after transplantation. The development of methods for determining whether a patient is becoming unresponsive to their transplant is critically important for future developments in transplantation. We think that a special population of white blood cells is responsible for controlling the immune response to the transplant. During the proposed project we will try to find out which cells are responsible for tolerance to a transplant and then develop methods for detecting the cells in transplant recipients. We hope that the information obtained from this study will allow strategies for monitoring the development of tolerance to the organ donor to be used to help transplant patients in the future.

Principle Investigator
Dr. Jiangping Wu

Notre-Dame Hospital, University of Montreal,
Montreal – Canada

Project:

To Improve Outcomes of Organ Transplantation by Inhibiting Function of a "Garbage Collector" Proteosome

The proteosome is a protein complex whose job is to degrade most of the proteins in a cell. It was considered as a "garbage collector" of cells, and its role in cells was thought humble and insignificant. Our recent work shows that function of the proteosome is tightly regulated and it plays critical roles in controlling cell growth and death. Notably, we have found that if activity of the proteosome is inhibited, cells that are vigorously growing will be easily killed, while cells that are not growing will be spared. This is a very interesting feature, and we will take advantage of it to improve outcomes of organ transplantation.

Organ transplantation is an effective way to treat various end-stage diseases of various organs. After organ transplantation, a graft will be rejected by a recipient because the immune system of the recipient will recognize the graft as a foreign entity. T lymphocytes of the immune system play a major role in the graft rejection. Some T cells are specific to foreign molecules (called alloantigen) of the graft and are stimulated by these molecules. These cells will start to grow and to proliferate and finally cause damage to the graft, while other irrelevant T cells that might be protective against microbes remain in a resting status. If we kill the growing T cells, then we can create a hole in the immune system such that it cannot reject the graft but its protective functions against other microbes remain intact. Our final objective is to use proteosome inhibitors to inhibit T cell proliferation and to kill alloantigen-specific T cells after organ transplantation with a view to controlling graft rejection and creating long-term graft tolerance. In this project, we will conduct a proof of concept study on the toxicity, pharmacokinetics and efficacy of a prototype proteosome inhibitor lactacystin in living mice. If these parameters are acceptable, we will then be able to proceed to achieve our final objective.

Principle Investigator
Dr. Robert Zhong
Dr. Anthony M. Jevnikar, Co-Applicant

University of Western Ontario, London – Canada

Project:
Developing New Agents for Organ Transplantation

Organ transplantation is the most desirable treatment for many patients with end-stage organ failure. Transplantation offers an excellent opportunity for rehabilitation, allowing patients to resume their normal lifestyle. However, patients after transplantation must take antirejection drugs for a lifetime, and there are shortcomings and disadvantages of chronic use of these drugs. These drugs lead to diminishing long-term graft survival, impact negatively on patient quality of life and increase significantly the long-term costs of transplantation. We propose to use a novel therapy to manipulate the mouse immune system to accept a foreign organ permanently without the long-term use of toxic drugs. Positive results will lead to preclinical nonhuman primate studies. Our final goal is to let patients accept organs without fear of rejection or complications from toxic immunosuppression.

To apply:

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