R O T R F

Roche Organ Transplantation Research Foundation

BIANNUAL REPORT

April 2000

The Roche Organ Transplantation Research Foundation

The mission of the Roche Organ Transplantation Research Foundation (ROTRF) is to advance the science of solid organ transplantation in order to improve the care of the thousands of patients undergoing transplantation every year. The results of the funded research projects will contribute to an understanding of many aspects of the clinical and scientific adventure of transplantation, e.g. the mechanisms of long-term organ deterioration, the consequences of tissue injury, and the opportunities to intervene in these processes.

The Foundation is an independent medical research charity that provides operating funds to established academic staff in universities, transplant centres and research institutes. The Foundation supports research in solid organ transplantation, particularly where there is an unmet medical need.

The funding, a donation from F. Hoffmann-La Roche Ltd., provides the Foundation with 25 million Swiss francs over the first five years. The funds are disbursed as grants of up to 300,000 Swiss francs distributed over three years. The Foundation is legally independent from F. Hoffmann-La Roche Ltd. and is guided solely by the Board of Trustees according to its charter.

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4. Progress Reports

1. Preface

The ROTRF is pleased not only to announce the grants awarded in its third and most recent funding cycle, but also to present the first progress reports of the first funding cycle.

The ROTRF was launched by the Roche Group in 1998 as an independent foundation, with the mission to support research projects aiming to advance the science of solid organ transplantation. Therefore, we, the ROTRF's Board of Trustees and the Scientific Advisory Committee (SAC), emphasize the need for innovation and novelty, and for thinking "outside the box." In keeping with this principle, the research proposals submitted to the ROTRF undergo thorough reviews by the SAC and are rated according to relevance to solid organ transplantation, originality and scientific excellence. The ROTRF strives to foster and nurture scientific creativity, the very essence of progress. Therefore, we strongly encourage researchers with innovative scientific ideas and approaches – also outside of the direct transplantation area – to apply themselves to transplant-related problems.

It is important to note that, although the supported 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. They might represent new leaps forward or may fuel other ideas or findings. Every step and promising hypothesis has to be tried and tested even if it eventually proves to be incorrect. It is usually a long way from the concept to the clinical application, even when the original hypothesis has been correct. We are convinced that the research projects funded by the ROTRF will contribute to gaining greater knowledge 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.

The Foundation is a great and unique achievement, and all involved can be proud of it. All those who have been involved in supporting the ROTRF to accomplish its mission deserve the Foundation's gratitude.

On behalf of the Board of Trustees

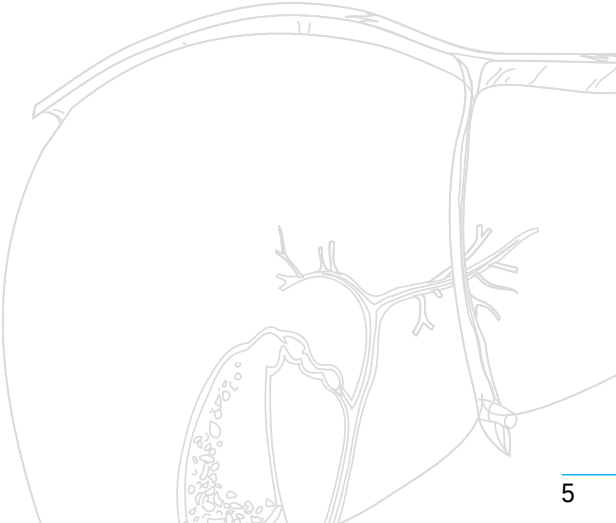
Phil Halloran

2. Facts and Figures

Funding Cycle III – Letter of Intent Submission in October 1999

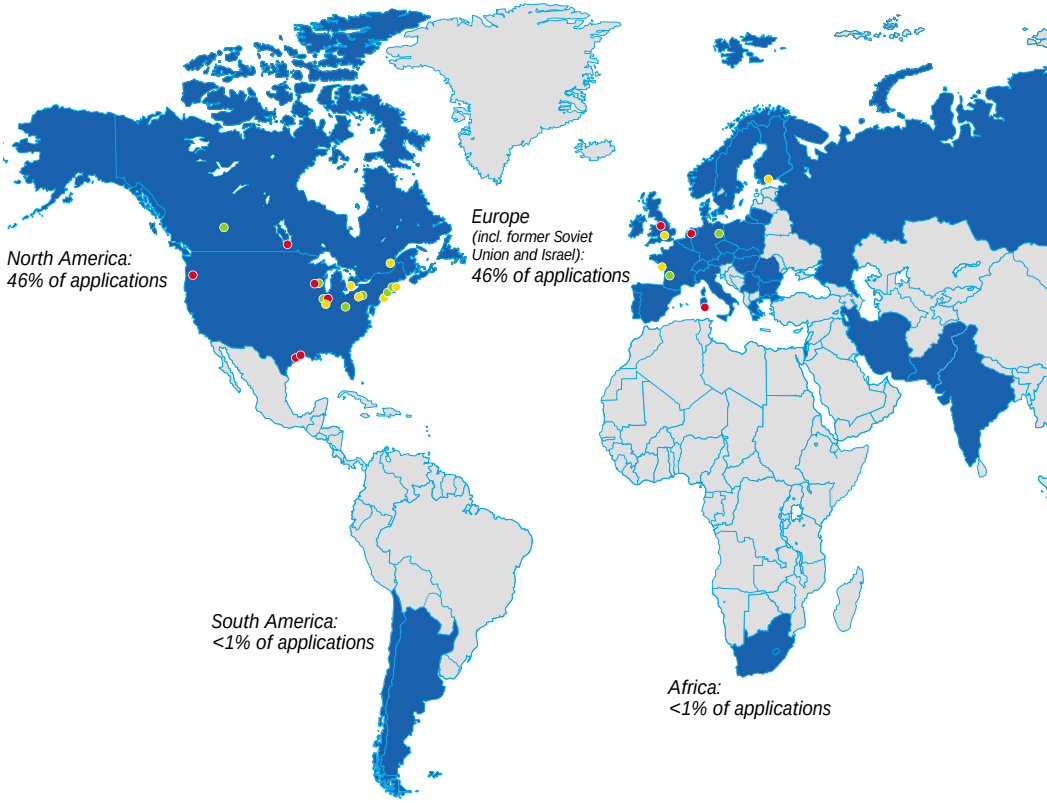
In this, the ROTRF's third funding cycle, nearly half (46%) of the applications were received from North America (USA and Canada) and 44% from Europe (UK constituted the majority with 16%). The remaining 10% of the applications were submitted from Australia, New Zealand and Asia.
The Scientific Advisory Committee of the ROTRF evaluated all of the applications that were submitted electronically via the ROTRF's homepage (www.ROTRF.org) for originality and scientific excellence. The top 20 applications were invited to submit a full paper application and subsequently underwent a second thorough review by the Scientific Advisory Committee and the Board of Trustees.
ROTRF grants were finally awarded to nine applicants, five from the USA, three from Europe (Italy, The Netherlands, United Kingdom) and one from Canada (please see the red dots on the world map on the following pages). They all presented excellent and very promising research projects (see abstracts on the following pages). Their research interests focus mainly on the improvement of long-term graft survival and prevention of chronic organ dysfunction, induction of tolerance, the development of new agents and relevant immune recognition, regulation and effector mechanisms.

In this third cycle of ROTRF grant awards, 1.8 million Swiss francs were disbursed in total.



The Global View of Applications to the ROTRF

Distribution of ROTRF applications worldwide



Cycle I
Grantees

Berlin, Germany
Bordeaux, France
Boston, USA
Chicago, USA
Cincinnati, USA
Edmonton, Canada
Madison, USA
Melbourne, Australia
New Haven, USA
Pittsburgh, USA

Cycle II
Grantees

Boston, USA
Chicago, USA
Helsinki, Finland
London, Canada
Montreal, Canada
Nantes, France
New York, USA
Oxford, UK
Pittsburgh, USA

Cycle III
Grantees

Birmingham, UK
Cagliari, Italy
Chicago, USA
Houston, USA
Houston, USA
Madison, USA
Nijmegen, The Netherlands
Portland, USA
Winnipeg, Canada

at least one application ever received
no application received

3. Grant Awards in Cycle III

Dr. Tausif Alam, Principle Investigator
Dr. Michael J. MacDonald, Collaborator
Dr. James S. Malter, Collaborator

University of Wisconsin, Madison – USA

Gene Therapy-Based Treatment for Insulin-Dependent Diabetes Mellitus

Insulin-dependent diabetes mellitus (IDDM) is caused by selective autoimmune destruction of insulin-producing β-cells of endocrine pancreas. Two therapies are currently available for IDDM, and both have serious limitations. The first therapy, based on daily insulin injections, inadequately controls hyperglycemia and consequently does not prevent the long-term damage associated with the disease. The second therapy, transplantation of whole pancreas or of pancreatic islets, precisely regulates blood sugar levels, but too few donor organs of suitable quality are available. Recent advances in cellular/molecular engineering now allow attempts for an alternative therapy by replacing the function of β-cell by a non-β-cell, engineered to provide insulin only when needed. For a precise gluco-homeostasis, β-cells instantaneously release stored insulin in response to high glucose levels. Therefore, the majority of studies attempting to generate a surrogate β-cell have focused on modifying cells that possess regulated secretory mechanism, but they have been unsatisfactory.

Our novel approach is based on recipient's own liver cells, engineered for glucose-regulated *de novo* synthesis and secretion of insulin, eliminating the need to duplicate a complex and incompletely understood mechanism of regulated insulin secretion of β-cells. The preliminary studies performed in cell culture and in diabetic rats provide ample proof of principle. The proposed optimizations will further improve the efficacy of transduced liver cells to normalize glucose levels of diabetic experimental rats. Using our approach, the diabetic recipients of insulin-gene-engineered hepatocytes may experience a transient, mild hyperglycemia in the minutes after eating, but should be able to avoid the chronic hyperglycemia that typically occurs with insulin injections alone, avoiding severe diabetes-associated complications. Success in our approach will provide a basis for the future gene therapy-based IDDM treatment that could be administered before the development of serious hyperglycemia-related complications. Furthermore, the use of autologous liver cells may eliminate the necessity of immunosuppression.

Dr. Irma Joosten, Principle Investigator
Dr. Luuk Hilbrands, Co-Applicant
Dr. Elly van de Wiel-van Kemenade, Co-Applicant

University Medical Center St. Radboud,
Nijmegen – The Netherlands

Cell Therapy to Prolong Graft Survival

Transplantation of organs (e.g. of hearts, lungs, livers and kidneys) is an important therapy for patients in whom the function of these organs has been lost. To prevent rejection of the transplant, immunosuppressive drugs are used that successfully suppress T-cell responses, but which have the common disadvantage that they also inhibit natural resistance against infections and malignancy. Furthermore, the different immunosuppressive drugs have a number of specific side effects. Consequently, new ways are being sought which allow for the induction of specific tolerance to the donor organ. In the past few years the attention has been directed to the inhibition of so-called costimulatory signals, important in T-cell activation. Recognition of (foreign) antigen by T-cells in the absence of a costimulatory signal results in the generation of so-called anergic T-cells. These cells are able to specifically suppress donor-directed immune responses. Prolonged graft survival has already been achieved by the *in vivo* administration of antibodies directed against these costimulatory molecules in animal models. However, administration of mouse or even humanised antibodies to a human transplant recipient has several drawbacks, such as non-specific effects on the immune system. We have now developed a system that allows us to generate anergic T-cells *ex vivo* with the aid of antibodies against costimulatory molecules. These anergic T-cells possess regulatory and so-called infectious properties, which means that the anergic state can be transferred to other T-cells, that in turn will become tolerant to the transplanted organ. In this project we want to study the *in vivo* immunosuppressive capacity of anergic T-cells using a mouse heart transplant model. To this purpose *ex vivo* anergised T-cells of a recipient mouse strain, tolerised against the donor organ prior to transplantation, will be infused at different time points around transplantation. These cells are expected to confer their immunosuppressive function in a donor-specific way. Particularly in combination with immunosuppressive drugs that allow tolerance induction we expect this treatment to be of potential use to human clinical practice, especially for organ (kidney) transplantations involving living (related) donors.

Dr. Robert A. Kirken, Principle Investigator
Dr. Stanislaw M. Stepkowski, Associate
Dr. Karras James, Collaborator
Dr. Federica Pericle, Collaborator

The University of Texas Health Science Center at Houston, Houston – USA

Identification of a Novel Protein Involved in Rejection of Transplanted Organs

There is an increasing need for successful transplantation of solid organs. Organ failure caused by complications to aging, autoimmune and inflammatory disease represents a rising health care problem and challenge to modern medicine. While major progress in organ transplantation has been achieved due to immunosuppressive drugs, their clinical value is limited due to associated toxicities. It is therefore a critical need to develop alternative strategies to achieve allograft acceptance. To provide a molecular rationale for innovative treatment strategies, new aspects of T-cell signaling must be explored. While current immunosuppressants (CsA and FK506) block T-cell receptor activation and interleukin-2 (IL-2) production, they are useless on T-cells that escape this regime, only to expand and promote chronic organ rejection. We propose to focus on novel signaling molecules downstream of the IL-2 receptor to block T-cell expansion. We reported that two related transcription factors, Stat5a and Stat5b, cannot be activated by IL-2 in T-cells isolated from immunocompromised tumor-bearing mice and HIV-infected patients, suggesting they are critical to T-cell regulation. In T-cells, we mapped IL-2-inducible tyrosine/serine phosphorylation sites in Stat5a/b and identified a novel pharmaceutical agent that blocked these pathways and consequently T-cell proliferation. Preliminary evidence suggests that temporary administration of this drug can promote rat heart transplant survival. Recent evidence that Stat5a/b knockout mice are immunosuppressed with T-cells unresponsive to IL-2 provides additional support for our model. Here, we will test the principal hypothesis that disruption of Stat5a/b signalling pathways prevents T-cell expansion and allograft rejection by three related specific aims designed to inactivate Stat5a/b by 1) our novel drug, 2) anti-sense and 3) dominant-negative gene variants delivered by viral and non-viral methods. At the conclusion of this research we expect to have established that inhibition of Stat5 transcription factors represents a viable therapeutic strategy to promote acceptance of transplanted organs.

Dr. Ezio Laconi, Principle Investigator
Dr. Annibale Donini, Co-Applicant
Prof. Paolo Pani, Associate
Dr. Sergio Laconi, Associate
Dr. Umberto Baccarini, Associate
Dr. Alberto Degrassi, Associate

University of Cagliari & Oncology Hospital
"A. Businco", Cagliari – Italy

Cell Transplantation to Treat Liver Disease

Organ transplantation is currently the only effective treatment for advanced liver disease. However, serious problems, including organ availability and high cost, severely limit its widespread clinical application. As a result, many candidate patients never reach access to this treatment. These considerations make it urgent to devise complementary or alternative approaches to whole organ replacement. To this end, transplantation of isolated liver cells is increasingly being investigated as a means to reestablish liver function in a diseased organ. Such a strategy would have several advantages, including lower cost, fewer complications and the possibility to use one donor liver for more than one recipient. The first human trials have in fact been reported, showing that transplanted liver cells can provide clinical benefit. However, a major limitation is that they do not grow in the recipient organ, unlike bone marrow cells, and this impairs their ability to fully restore liver function. Overcoming this limitation would certainly pave the way towards a broader applicability of liver cell transplantation as a therapeutic strategy.

In this context, we have recently developed an experimental model, which can serve as a general system for the expansion of transplanted cells in the liver. Near-total liver replacement by transplanted normal cells was observed in rats whose endogenous cells were blocked by exposure to a drug. Further studies indicated that transplanted cells can support normal liver function and have the ability to correct metabolic alterations when injected into genetically deficient animals. The clinical significance of these findings is enormous. Our current efforts are oriented to: (1) optimize the conditions for maximum therapeutic efficacy of this novel approach to liver cell transplantation; (2) exploit the potential of this strategy as a means to expand human liver cells in a different species; harvesting these cells would then provide an unlimited source of human cells for the treatment of liver disease.

Dr. Kenneth A. Newell, Principle Investigator
Dr. Yun Wang, Associate

University of Chicago, Chicago – USA

Effect of T Cell Costimulatory Blockade and Bone Marrow Transplantation on Organ Transplant Rejection

Rejection remains a major barrier to organ transplantation. This is particularly true for transplanted intestines. T cells play a critical role in rejection. In order for T cells to mediate rejection they must undergo activation. This process is dependent upon two signals: one resulting from the recognition of foreign antigens from the transplanted organ and another signal commonly referred to as a costimulatory signal. T cells receiving the first signal without the costimulatory signal become unresponsive and often die. Thus, if T cells were allowed to receive the first signal from a transplanted organ while the costimulatory signal was blocked, these T cells might die and rejection might be avoided. Using this strategy several investigators have shown that blocking costimulatory signals at the time of transplantation resulted in the long-term survival of transplanted organs in numerous rodent and preclinical primate models. Two of the major costimulatory pathways are the CD28/B7 pathway and the CD40/CD154 pathway. We compared the effect of blocking these pathways on the rejection of transplanted intestines and hearts in mice. Although blocking either of these costimulatory pathways prevented heart rejection, no effect was seen on rejection of intestines. We have shown that this difference is caused by a subset of T cells characterized by the expression of the CD8 molecule on their surface. Our findings demonstrate that these $CD8^+$ cells are uniquely important for the rejection of intestine allografts and that rejection caused by $CD8^+$ cells can not be inhibited by blocking either the CD28 or the CD40 pathways. Recently, it has been shown that transplanting bone marrow cells augments the effects of blocking costimulatory pathways. We hypothesize that by combining bone marrow transplantation and costimulatory blockade, the rejection of transplanted intestines by $CD8^+$ T cells can be inhibited. If confirmed, this approach may be applied clinically to improve the outcome of transplanted intestines and other organs.

Dr. Peter W. Nickerson, Principle Investigator
Dr. Ray Somorjai, Co-Applicant
Dr. Roxanne Deslauriers, Co-Applicant
Dr. David Rush, Co-Applicant

University of Manitoba, Winnipeg – Canada

Non-Invasive Diagnosis of Acute Renal Allograft Rejection

This proposal seeks to determine whether an informatics approach using computer-generated, multivariate classification strategies of urine magnetic resonance (MR) or infrared (IR) spectra can be used to diagnose acute renal allograft rejection. If successful, urine MR/IR spectra may serve as a monitoring technique in acute/chronic rejection trials, or tolerance protocols. Moreover, such a novel means for repetitive non-invasive, outpatient assessment of graft inflammation may improve graft outcome as therapy could be tailored to the individual, thereby avoiding both excessive and insufficient immunosuppression while minimizing the risks associated with invasive post-transplant monitoring tests (i.e. protocol biopsy).

Dr. Susan L. Orloff, Principle Investigator
Dr. Daniel N. Streblow, Co-Applicant
Craig Kreklywich, Associate
Dr. Qiang Yin, Associate

Oregon Health Sciences University, Portland – USA

Role of Viral Chemokine Receptors in Cytomegalovirus-Accelerated Transplant Vascular Sclerosis

The primary cause of graft loss of all vascularized organ transplants is due to a vascular lesion associated with chronic rejection. This form of vasculopathy, referred to as transplant vascular sclerosis (TVS), is primarily characterized by concentric neointimal smooth muscle cell proliferation that results in vessel occlusion and ultimately graft failure. To date the only therapy available to treat severe TVS is re-transplantation.

Cytomegalovirus (CMV) is a ubiquitous virus that is carried in a latent form by most individuals (60–75% of the population), and is known to accelerate TVS, although through undefined mechanisms. Recently, we demonstrated that during human CMV infection of smooth muscle (SMC) the CMV-encoded chemokine receptor, US28, induces SMC migration *in vitro.* Rat and murine CMV also induce SMC migration of infected cells, mediated by their respective viral chemokine receptor homologues, R33 and M33. Based on our preliminary results, we hypothesize that the mechanism of CMV-accelerated TVS involves the expression of virally encoded chemokine receptors leading to intimal SMC migration, which results in the characteristic vascular lesions of TVS. Using a rat cardiac transplant model of chronic rejection we will determine the kinetics of disease progression of TVS, the extent of viral expression in tissues, the cell types and the host factors such as chemokine cytokines, and growth factors involved at various stages of the development of RCMV-induced TVS. We will then determine the effects of deletion of the CMV-encoded chemokine receptors on CMV-accelerated TVS in this cardiac transplant model. These studies will provide the molecular link to the role of CMV in the acceleration of TVS, and will serve as the basis for novel and rational design of therapeutic strategies to enhance long-term graft survival in the majority of transplant recipients, who are latently infected with CMV.

Dr. Stanislaw M. Stepkowski, Principle Investigator
Dr. Min Wang, Co-Applicant
Dr. Robert A. Kirken, Consultant
Dr. Lin Tian, Associate

The University of Texas Health Science Center at Houston, Houston – USA

Cytokine-Activated Signalling in T Cells is Required for Tolerance Induction by Allochimeric Protein

Continual treatment of patients with current immunosuppressive drugs has dramatically improved allograft survival rates, with only a minority of patients today suffering from acute rejection. However, the vast majority of transplant patients do develop chronic rejection, which results in impaired graft function or even patient death. To remedy this outcome is to induce a state of permanent acceptance of allografts; namely, transplantation tolerance (TT). As recently shown by other investigators, the selective blockade of B7/CD28 second signals induced TT by increasing activation-induced cell death (AICD) among alloreactive T cells. Rapamycin (RAPA) increased AICD and facilitated induction of TT, while cyclosporine (CsA) prevented both AICD and induction of TT, documenting that active AICD is not necessary for TT.

In our model, TT was induced by modulation of the first TCR signal using donor/recipient class I major histocompatibility complex allochimeric protein. A single portal vein injection or multiple oral gavage with allochimeric protein alone (but not unmodified donor RT1.A^{u} antigen) induced donor-specific TT. The state of TT is transferable with T cells that display increased expansion of IL-4-producing T helper type 2 (Th2) cells. In contrast to immunogenic RT1.A^{u} protein, allochimeric protein induces only partial activation (tyrosine phosphorylation of Zap70 kinase) in RT1.A^{u}-specific T cells. Since CsA facilitated induction of TT by allochimeric protein, we postulate that AICD is not necessary in our model. However, either of two cytokine-receptor signal inhibitors (RAPA or AG490) abolished induction of TT. Therefore, we plan to show that allochimeric protein induces TT without active participation of AICD. Also, we plan to show that incomplete TCR activation requires effective second signal and a continuous activation of the signal transducer and activator of transcription Stat6, to constantly up-regulate Th2 cells. Thus, TT may be induced and maintained without AICD, but with regulation of IL-4-driven Stat6 pathway.

Dr. Deborah M. Stroka, Principle Investigator
Dr. Daniel Candinas, Co-Applicant

University of Birmingham, Birmingham – UK

The Role of Oxygen in Transplant Rejection

Chronic allograft rejection following kidney, heart and liver transplantation represents the major cause of late graft failure. The pathogenesis of this clinically important problem is incompletely understood and warrants further investigations. The aim of our current work is to determine if hypoxia is an important factor in this process. In order to investigate the role of hypoxia in late organ loss, we are producing a transgenic mouse that carries a transgene that is activated only under conditions of decreased oxygen. This hypoxia-sensitive reporter mouse will detect low tissue oxygen levels and will be used in a heart transplant model to study the presence of tissue hypoxia in correlation with the process of chronic rejection. We will further investigate the role of hypoxia in the various cell types involved in chronic rejection, such as smooth muscle cells, endothelial cells and macrophages. These studies may identify hypoxia not only as an important regulating factor, but also a predictive marker in late stage graft loss. If our hypothesis proves to be correct, a long-term goal is to specifically regulate hypoxia-driven responses in order to intervene and to reverse the transplant rejection process.

4. Progress Reports

Prof. Anthony J. F. d'Apice, Principle Investigator
Dr. Peter J. Cowan, Co-Applicant
Dr. Trixie A. Shinkel, Co-Applicant

St. Vincent's Hospital Melbourne, Melbourne – Australia

Prevention of Rejection of Organ Transplants between Species by Anti-Oxidant Genes

Preparation of Expression Constructs and *in Vitro* Testing

Transient Transfection: The cDNAs for native and epitope-tagged forms of copper zinc superoxide dismutase (CuZn-SOD), glycosyl-phosphatidyl inositol-linked extracellular superoxide dismutase (GPI-linked EC-SOD) and glutathione peroxidase (GPx) have been subcloned into the mammalian expression vector pcDNA3 and transiently transfected into COS cells. Expression was detected by flow cytometric analysis (FACS) of intact (GPI-linked EC-SOD) or saponin-permeabilised (CuZn-SOD) cells using specific antibodies to the antioxidant enzymes and/or to the epitope tags. High levels of human CuZn-SOD and EC-SOD expression were detected. Untagged and tagged versions of both CuZn-SOD and GPI-linked EC-SOD produced comparable levels of expression. Transient transfections of GPx (tagged and untagged) into COS cells are in progress.

Enzyme Activity Assays: SOD activity of cell homogenates has been assayed spectrophotometrically using an activity assay based on the procedure described by Elstner and Heupel (1976) and modified by Oyanagui (1984). Cell homogenates from transient transfections showed measurably increased activity compared to control cells. Untagged and tagged versions of both CuZn-SOD and GPI-linked EC-SOD produced similar levels of activity.
GPx activity of cell homogenates will be assayed spectrophotometrically using the coupled assay procedure of Paglia and Valentine (1967). This assay has been optimised using purified GPx from human erythrocytes.

Because tagging of the enzymes was shown to have no detrimental effect on expression or activity, the tagged versions will be used for all subsequent experiments as this will simplify their detection in transfectants and in transgenic animals.

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

University of Cincinnati, Cincinnati – USA

Molecular Regulation of Liver Cell Transplantation

Specific Aim: To define the role of the plasminogen activator family of proteins on engraftment and expansion of transplanted hepatocytes.

The main focus of our work during this first year has been:
1. Assessment of the impact of plasminogen and plasminogen activators on the reparative response of the liver to an acute injury. This was done in the mice lacking specific proteases, which will be used as donors and recipients in transplantation experiments.
2. The generation and phenotypic characterisation of the mouse model to be used as donor of hepatocytes in transplantation experiments.

Studies and Results: In order to fully establish the impact of plasminogen activators on the fate of transplanted hepatocytes, we first completed a series of experiments to define how plasminogen and its activators urokinase-type (uPA) and tissue-type (tPA) plasminogen activators regulate the reparative response of the liver to an acute injury. These initial steps are important in order to understand the results of transplantation experiments to be performed in the upcoming year. The initial series of experiments were divided in two phases.

1. Role of plasminogen in liver repair
We used mice that carry the targeted inactivation of the gene coding for plasminogen in a well-established experimental model of liver injury. Plasminogen is an important serine protease which controls fibrinolysis during clotting and the reparative response of extra-hepatic tissues to an injury. In our experiments, we injected carbon tetrachloride (CCl_4), a liver-specific toxin, into mice lacking plasminogen (Plg^0) and control littermates (Plg^+). Two days after CCl_4-treated livers of Plg^+ and Plg^0 mice displayed similar diseased pale/lacy appearance, followed by restoration of normal appearance in Plg^+ liver by 7 d. In contrast, Plg^0 livers remained diseased for as long as 2.5 months, with a diffuse pale/lacy appearance and a persistent damage to centrilobular hepatocytes. The persistent centrilobular lesions were not a consequence of impaired proliferative response in Plg^0 mice.

Notably, the deposition of fibrin and other extracellular matrix substrates was a prominent feature in diseased centrilobular areas in Plg^{0} livers for at least 30 d after injury. Together, these data demonstrate that plasminogen deficiency results in defective repair following an acute liver injury. The accumulation of matrix substrates in Plg^{0} livers also points to a regulatory role of plasminogen in the proteolytic reorganization of the hepatic lobule which occurs after an injury. These studies have been completed and were published in a recent issue of the Journal of the Proceedings of the National Academy of Science (Bezerra at al., 1999a).

2. Role of plasminogen activators in liver repair

Based on the findings of defective repair in mice lacking plasminogen, we used the same experimental protocol in mice carrying the targeted inactivation of the genes coding for uPA and tPA. Analysis of preliminary data demonstrates that tPA deficiency causes a mild defect in the reparative response of the liver to an acute injury, while uPA deficiency causes a moderate defect. Interestingly, the combined loss of tPA and uPA results in a severe defect in liver repair in a fashion similar to that observed in mice lacking plasminogen. Although these studies are still in progress, we speculate that tPA and uPA work synergistically to activate plasminogen, which promotes liver repair after an acute injury. These preliminary data were presented as a poster in the annual meeting of the American Association for Studies of Liver Disease in Dallas, TX, USA (Bezerra et al., 1999b).

The studies described above clearly establish that plasminogen activators and plasminogen play a regulatory role in the proteolytic modification of matrix substrates during an acute liver injury. These unique mouse models will then be used in hepatocyte transplantation experiments to explore whether each protease facilitates engraftment and expansion of transplanted cells in the liver through proteolysis of matrix substrates in the microenvironment surrounding transplanted hepatocytes. In order to proceed with transplantation experiments, we have also worked on the expansion of a colony of genetically mutant mice, which overexpress uPA and lack plasminogen (uPA^{+}/Plg^{0}). By creating these mutant mice, we have produced a unique *in vivo* setting in which we can directly separate the biological consequences of uPA from plasminogen. Such a separation is very important for the potential use of uPA or plasminogen before or after transplantation experiments to optimize the population of transplanted hepatocytes in the recipient livers and improve the outcome of transplanted subjects.

Preliminary analysis of ongoing studies suggests that mutant uPA^{+}/Plg^{0} mice have a normal liver phenotype, as demonstrated by normal gross appearance and histological pattern of liver sections stained with hematoxylin-eosin. We are currently performing electron microscopy to explore the existence of any ultrastructural abnormalities, which may result from the genetic alteration of these mice. The complete phenotypic characterisation of uPA^{+}/Plg^{0} mice is important because these mice will be used as donors of hepatocytes in a series of transplantation experiments outlined in the original grant proposal.

Significance: The use of gene-targeted mice in experimental models of acute liver injury clearly demonstrates that plasminogen and plasminogen activators play an essential role in the reparative response of the liver to an injury. In our studies, the loss of any of these proteases leads to an abnormal accumulation of matrix substrates and a defective reorganization of the liver lobule. In upcoming experiments, we will directly explore how plasminogen/plasminogen activator-mediated matrix proteolysis direct the biological fate of transplanted hepatocytes *in vivo*.

Publications

Bezerra JA, Bugge TH, Melin-Aldana H, Sabla G, Kombrinck KW, Witte DP, Degen JL. Plasminogen deficiency leads to impaired remodeling following a toxic injury to the liver. Proc Natl Acad Sci USA 1999a; 6:15143–15148 (original article).

Bezerra JA, Bugge TH, Melin-Aldana H, Sabla G, Kombrinck KW, Witte DP, Degen JL. Plasminogen activators direct matrix remodeling following an acute liver injury. Hepatology 1999b; 30:490A (abstract).

Ng VL, Alonso M, Bezerra JA. Hepatocyte transplantation: advancing biology and treating children. Clin Liv Dis, submitted for publication (invited review article).

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

University of Chicago, Chicago – USA

Immunosuppression and Tolerance Induction by Genetically Engineered Dendritic Cells Expressing Cell-Surface Anti-CTLA-4 mAb

Experimental efforts during the first year of funding have focused on optimising the functional efficacy of surface-linked anti-CTLA-4 single-chain antibody (scFv) and on generating protocols for the efficient transfer of this construct to murine dendritic cells (DCs). Our studies have revealed that surface expression of the parent scFv is limited following stable gene transfer as a result of structural instability of the antigen-binding domain.
We have addressed this, in collaboration with Dr. David Kranz (University of Illinois, Urbana, IL, USA), by using a mutagenesis/yeast-display strategy to select mutated forms of surface-linked anti-CTLA-4 scFv. In this way, we have generated and characterised a surface-linked mutant scFv (manuscript under review) that exhibits significantly increased affinity (approximately 1000-fold) and greater stability. This construct has been successfully transferred to DCs by retroviral transfection as described for specific aim #2 (use of a retrovirus-based gene transfer system to engineer anti-CTLA-4-expressing APCs for tolerance induction).

In ongoing experiments being carried out by Dr. William Sweatt (University of Chicago) and Dr. Griffin (Mayo Clinic, Rochester, MN, USA) the immunomodulatory function of scFv-expressing P815 tumor cells and DCs is being characterised as outlined for specific aim #3 (comparison of the effects of mem4F10scFv-expressing DCs and control DCs in allotransplantation).

Initial studies with the P815 cells have demonstrated a 2–8-fold enhancement of immunosuppressive activity with the high affinity mutant anti-CTLA-4 scFv. With the development of a functionally enhanced construct, the generation of transgenic mice expressing anti-CTLA-4 scFv for use in models of transplantation (specific aim #1, the generation of transgenic mice expressing a surface-linked CTLA-4-specific ligand on DCs) will be carried out in Dr. Bluestone's laboratory during years 2 and 3 of funding.

We have also initiated the development of an adenoviral vector for transient, high-level expression of the mutated scFv in quiescent cell populations such as pancreatic islets. This approach will allow additional, clinically relevant transplantation strategies to be evaluated.

Prof. Alfred L. M. Bothwell, Principle Investigator

Yale University School of Medicine,
New Haven – USA

Identification of Novel Porcine Immunoregulatory Molecules

The aims of this grant are the isolation and characterisation of known as well as unknown immunoregulatory molecules from porcine aortic endothelial cells (PAEC). Two methodologies are being applied: representation difference analysis (RDA) and generation of monoclonal antibodies.

We have chosen to use primary PAEC cells first for the RDA analysis because it represents a more physiologic cell type rather than the SV40-immortalised line PEC-A that we derived. We are routinely isolating fresh PAEC so there is not a limitation in the source of cells. We have begun the RDA studies with a single isolate and made RNA from untreated cells, cells treated for 16 hr with porcine IFN-γ and cells treated for 4 hours with human TNF-α. The PCR-based RDA analysis is ongoing, and DNA fragments isolated from this strategy will be sequenced in the near future. Full length cDNAs and myc epitope-tagged forms of porcine cell interaction molecules have been constructed (CD40, VCAM, E-selectin and P-selectin). During the first year we have also made observations that may broaden the scope of this investigation. The isolated PAEC have been cast in 3-dimensional collagen-fibronectin gels and an extensive network of microvessels form within 24 hours. These gels have also been implanted into SCID mice where extensive connections with mouse microvessels and the porcine microvessels are made resulting in perfusion of the vessels with mouse blood. Retroviral transduction of cells has resulted in PAEC that show recruitment of murine smooth muscle cells *in vivo.* Therefore, we are interested in performing the RDA analysis on the transduced PAEC versus control vector transduced PAEC to identify the important differences.

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Study of the Role of Gamma-Delta T Lymphocytes in the Immune Response Directed against Human Cytomegalovirus

The main aims of our research project are to understand how γδ T lymphocytes are implicated in the immune response directed against CMV and to identify the yet unknown ligands of these peripheral Vδ1 and Vδ3 T cells. The first significant progress we made since last year was the generation of cell lines and clones from γδ T cells isolated from CMV-infected transplanted patients. All of these purified γδ T cells display cytotoxic activity when polyclonally activated, and some are able to react against CMV-infected but not against uninfected fibroblasts. Study of TCR implication in this process, and identification of the putative CMV or CMV-induced antigens recognised by γδ T cell clones are currently progressing. As a useful tool for the ligand search, we will also generate a soluble form of a Vδ1 TCR from a clone strongly expanded in a CMV patient and displaying *in vitro* reactivity against CMV and the previously observed selection of W and P amino-acid motifs in the CDR3 region. Besides, we further extended the implication of γδ T cells in CMV-infection to liver- and lung-transplanted patients, and more importantly, to CMV-seropositive healthy individuals who display enhanced Vδ1 T cell percentage in peripheral blood when compared to CMV seronegative individuals. Comparison of Vδ1 and Vδ3 T cell repertoire between seropositive and seronegative healthy donors is under investigation by sequencing the CDR3 regions of the δ chain. Finally, a prospective study in a cohort of 64 transplanted patients developing CMV infection showed that a delay in γδ T cell expansion is predictive of severe and prolonged CMV infection.

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The Role of Senescence and Telomere Shortening in Chronic Rejection

Aim of the Study: To examine cell aging ('senescence') mechanisms in transplantation. Our hypothesis has been that kidneys age by mechanisms similar to 'replicative senescence' in culture, and that stresses of transplantation aggravate these changes.

Results: The focus of our work during the first year of support has been on the importance of telomere shortening and of genes responsible for regulating senescence for human and rodent kidneys.

We explored the effect of aging on telomere length in 47 surgical or autopsy kidneys which were either histologically normal (N=34) or displayed histologic abnormalities (N=13). Southern blotting of terminal restriction fragments (TRF) and slot blotting with a telomere-specific probe were performed. Mean telomere length in the renal cortex decreased with age, shortening by about 0.024 kb per year (0.20%). Assessment of telomere DNA in cortex by slot blot revealed a loss with age at a similar rate (0.23% per year). The mean telomere length was not different between cortex and medulla (mean 10.43±1.36 kb versus 10.42±1.16 kb, p=0.75). When younger kidneys were analyzed the difference in telomere length between cortex and medulla was greater but diminished with age due to greater telomere loss in cortex. The rate of telomere loss was not significant in medulla by either TRF or slot blots. Calculated GFR (Cockcroft-Gault) in these patients declined with age as expected (1.3% per year over 30, p=0.0003) but the correlation between telomere length and GFR was weak (not significant). These findings are compatible with a role for telomere shortening and replicative senescence in aging in renal cortex and suggest that cells in medulla and cortex have different replicative histories in development and in aging. Telomere length could potentially influence the ability of the kidney to respond to injury and disease, but the actual significance of age-related telomere loss and other genomic changes in the age-related pathology remains to be established.

The cyclin-dependent kinase inhibitor $p16^{INK4a}$ is a mediator of cellular senescence *in vitro*. It plays a key role at the G1 cell cycle checkpoint by inhibition of CDK4 and CDK6, thereby blocking cell proliferation. Measurement of $p16^{INK4a}$ mRNA expression was done using quantitative RT-PCR (TaqMan® technology). Gene expression was measured in mouse kidney samples following injury with the nephrotoxin, cisplatin, or ischemia. In addition, we investigated human kidney samples derived from individuals from different ages (normal histology: N=17, abnormal histology: N=5). $p16^{INK4a}$ was not expressed in normal mouse kidneys (age 6–8 weeks) and could not be induced by injury. In human kidney samples derived from individuals under the age of 30 years we found low $p16^{INK4a}$ expression. Some kidneys from older individuals (age 41–88 years) did express significant $p16^{INK4a}$ mRNA in cortex ($3.7x10^{-4}$, $3.5x10^{-4}$, $2.8x10^{-4}$), although expression was quite variable. High levels of $p16^{INK4a}$ expression were also observed in abnormal kidneys derived from individuals with hydronephrosis, interstitial nephritis, pyelonephritis and adult polycystic kidney disease. $p16^{INK4a}$ tend to increase with age, although this increase did not reach the level of significance (p=0.14). This data suggests the hypothesis that p16 is not normally expressed in young kidney or inducible by injury, but is induced with aging and disease, compatible with its putative role in cellular senescence.

We are currently carrying out two kinds of injury experiments in a wild mouse strain, *Mus spretus:* Ischemic acute tubular necrosis (ATN) and toxic renal injury by gentamicin and cisplatin. We will then assess the *in vivo* changes of telomere length, telomerase TERT mRNA and TR mRNA as well as in senescence-regulating genes such as $p16^{INK4a}$. We established the use of pulse field gel electrophoresis (PFGE) for investigation of telomere restriction fragments with an average length of 50 kb in laboratory rodents. Using this technique we are currently investigating kidney samples derived from normal rats and mice at different ages (1, 9 and 24 month) as well as after different types of renal injury.

In summary, we have begun all aspects of the project. We have demonstrated telomere shortening in kidney cortex with age as a 'proof of concept', and have promising results for $p16^{INK4a}$.

References and Publications

Halloran PF, Melk A, Barth C. Rethinking chronic allograft nephropathy – the concept of accelerated senescence. J Am Soc Nephrol 1999; 10:167–181 (Review).

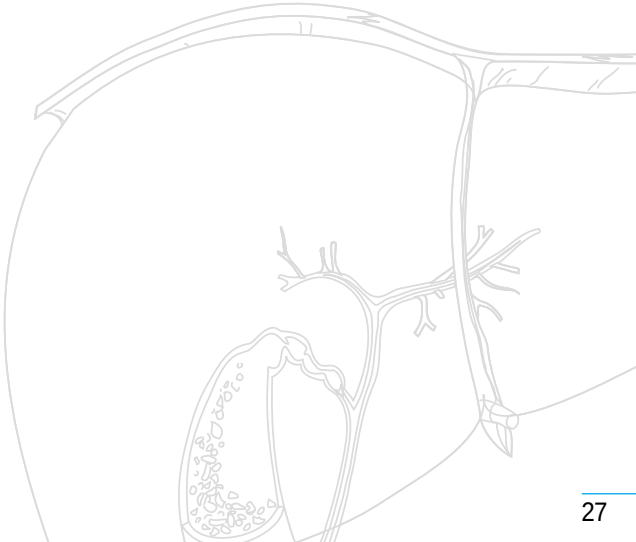
Melk A, Ramassar V, Halloran PF. Telomeres shorten in the aging kidney – is this the key to chronic allograft nephropathy? 9th Annual ESOT Meeting 1999, Oslo, Norway (Abstract).

Melk A, Ramassar V, Helms LM, Moore R, Rayner D, Solez K, Halloran PF. Telomere shortening in kidneys with age. J Am Soc Nephrol 2000; 11:444–453 (Article).

Melk A, Helms L, Moore R, Rayner D, Solez K, Halloran PF: Loss of telomere DNA in renal cortex with age. J Am Soc Nephrol 1999; 10:82A (Abstract A0420).

Melk A, Ramassar V, Halloran PF. Telomere shortening in human kidney: the key to the adverse effects of donor age? Transplantation 1999; 67(7): S.252 (Abstract 983).

Melk A, Turner P, Helms LMH, Ramassar V, Urmson J, Halloran PF. Expression of senescence regulator $p16^{INK4a}$ mRNA in some human kidney samples (Abstract submitted to ASN 33rd Annual Meeting, October 12–16, 2000).



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Heme Oxygenase-1: an Anti-Inflammatory Molecule that Promotes Organ Graft Survival

We have previously shown that mouse-to-rat cardiac transplants can survive in the long-term under brief inhibition of complement activation and continuous T cell immunosuppression (Koyamada et al., 1998). Expression of the protective gene heme oxygenase-1 (HO-1) by graft endothelial and smooth muscle cells is essential to insure graft survival (Soares et al., 1998; Soares et al., 1999). We have now tested whether the ability of HO-1 to sustain graft survival relies on its ability to catabolize heme into the gas carbon monoxide (CO). Our present data suggest that this is the case. Under the same immunosuppressive regimen that allows mouse-to-rat cardiac grafts to survive in the long-term, inhibition of HO activity by tin protoporphyrin (SnPP) precipitated graft rejection in 3–7 days. The pathological features of graft rejection under inhibition of HO activity by SnPP were very similar to the ones observed in HO-1-deficient mouse hearts (Soares et al., 1998). In both cases there was massive myocardial infarction associated with infiltration by monocytes/macrophages (Mø), thrombosis and apoptosis of endothelial cells (EC) as well as myocytes. Exposure of the recipient to exogenous CO prevented graft rejection and restored long-term graft survival despite inhibition of HO-1 activity. The ability of CO to prevent graft rejection was associated with inhibition of infarction, Mø infiltration, thrombosis and EC apoptosis. These data suggest that the protective effect of HO-1 in preventing graft rejection is mediated through the generation of CO. We have further investigated the mechanism underlining this protective effect by testing whether CO would directly inhibit platelet aggregation, Mø activation and/or EC apoptosis *in vitro.* Our present data suggest that this is the case. Exposure of platelets to EC expressing high levels of HO-1 suppressed platelet aggregation. Exposure of Mø to CO suppressed the pro-inflammatory phenotype associated with Mø activation (Otterbein et al., 2000). Exposure of EC to CO suppressed EC apoptosis. Taken together these observations provide a mechanistic explanation for the ability of HO-1/CO to suppress graft rejection.

Publications and References

1. Koyamada N, Miyatake T, Candinas D, Mark W, Hechenleitner P, Hancock WW, Soares MP, and Bach FH. Transient complement inhibition plus T-cell immunosuppression induces long-term survival of mouse-to-rat cardiac xenografts. Transplantation 1998; 65, no. 9:1210–1215.

2. Soares MP, Lin Y, Anrather J, Csizmadia E, Takigami K, Sato K, Grey ST, Colvin RB, Choi AM, Poss KD, and Bach FH. Expression of heme oxygenase-1 (HO-1) can determine cardiac xenograft survival. Nature Medicine 1998; 4: 1073–1077.

3. Soares MP, Lin Y, Sato K, Stuhlmeier KM, and Bach FH. Accommodation. Immunology Today 1999; no. 10:434–437.

4. Otterbein LE, Bach FH, Alam J, Soares M, Tao HL, Wysk M, Davis R, Flavell R, and Choi AMK. Carbon monoxide mediates anti-inflammatory effects via the mitogen activated protein kinase pathway. Nature Medicine 2000; no. 4:422–428.

Prof. Angus Thomson, Principle Investigator
Dr. Adrian Morelli, Co-Applicant
Dr. Petra O'Connell, Co-Applicant
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Studies of a Novel Dendritic Cell (DC) Population in Organ Allograft Survival

Our studies during the first year of the award have focussed on further characterization of the role of lymphoid(-related) dendritic cells in the induction and regulation of alloimmune responses, including organ transplant rejection in the mouse. Methods have been refined for the isolation and purification of these cells from both lymphoid and non-lymphoid tissues. We have characterized extensively the surface immunophenotype and stimulatory ability (for naive T cell activation) of freshly isolated lymphoid DC, and of lymphoid DC matured overnight in culture with GM-CSF. We have established that mature lymphoid DC, like mature myeloid DC, are powerful inducers of allogeneic naive T cell proliferation and preferentially induce Th1 responses, as determined by intracellular cytokine staining of $CD4^+$ T cells. Immature lymphoid DC, however, are poorer inducers of T cell proliferation than immature myeloid DC, and induce comparatively high levels of apoptotic death in alloactivated $CD4^+$ and $CD8^+$ T cells, as determined by TUNEL staining and flow cytometric analysis. This property suggests a possible mechanism whereby lymphoid DC may be able to regulate alloimmune reactivity.

An important property of classic myeloid DC is their capacity to migrate from peripheral tissue sites to T cell areas of secondary lymphoid tissue, as occurs following organ transplantation. Although one recent report suggests that lymphoid DC may not share this ability with myeloid DC, our immunohistological and molecular biologic observations clearly indicate that allogeneic lymphoid DC do have the capacity to traffic *in vivo* to T cell areas of lymph nodes and spleen. Moreover, mature (overnight-cultured) lymphoid DC, like myeloid DC, prime allogeneic T cells *in vivo* as shown by *ex vivo* responsiveness to donor alloantigens in MLR. Of considerable significance is our recent finding that systemic (iv) injection of

highly purified, donor-derived mature lymphoid DC 7 days before transplant can significantly and markedly prolong vascularized organ (heart) allograft survival in the mouse (B10 [$H2^b$]→C3H [$H2^k$] strain combination). By contrast, and as anticipated, mature myeloid DC accelerate graft rejection. This is the first observation of which we are aware that reveals a regulatory function of lymphoid DC, in the host response to an organ allograft.

This important novel observation will be reported by Dr. O'Connell (oral presentation) at the upcoming (May 2000) joint meeting of the American Society of Transplantation and the American Society of Transplant Surgeons in Chicago IL. The work has also been accepted for oral presentation at the 6th International Dendritic Cell Symposium in Port Douglas, Australia (May 2000), and at the International Transplantation Congress, Rome, August 2000.

Our work during the upcoming second year of the award will focus on elucidating the mechanism(s) underlying the capacity of lymphoid DC to inhibit organ allograft rejection. In view of findings made during year one of the award, we shall focus on the role of apoptosis in regulation of the host T cell response.

Publications and Presentations

O'Connell PJ, Morelli AE, Logar AJ, and Thomson AW. Phenotypic and functional characterization of mouse hepatic $CD8\alpha^+$ lymphoid-related dendritic cells. J Immunol 2000; *in press* (Peer-reviewed manuscript accepted for publication).

Morelli AE, O'Connell PJ, Khanna A, Logar AJ, Lu L, and Thomson AW. Preferential induction of Th1 responses by functionally mature hepatic ($CD8\alpha^-$ and $CD8\alpha^+$) dendritic cells: Association with conversion from liver transplant tolerance to acute rejection. Transplantation 2000; *in press* (Peer-reviewed manuscript accepted for publication).

Faratian D, Colvin BL, O'Connell PJ, Morelli AE, Thomson AW. Dendritic cell heterogeneity – a complex picture emerges. Graft 2000; 3: 54–58 (Review).

Colvin BL, Faratian D, O'Connell PJ, Morelli AE, Thomson AW. Dendritic cell ontogeny. Graft 2000; 3: 96–97 (Review).

Thomson AW. A critical look at the antigen-presenting capacity and tolerogenic potential of dendritic cells: What is the *in vivo* evidence that is important? Graft 2000; *in press* (Review).

Abstracts were and have been accepted for presentation at international meetings, such as at the AST (Chicago, IL, USA), the 6th International DC Symposium (Port Douglas, Australia), the International Transplantation Congress (Rome, Italy) and the 6th Basic Sciences Symposium of the Transplantation Society (Asilomar, CA, USA).

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Gene Expression of Tolerance-Mediating Allospecific Cells

1. We investigated the importance of antiapoptotic proteins (e.g. Bag-1) for the generation of long-living regulatory T cells and the maintenance of allograft tolerance by the following experiments:

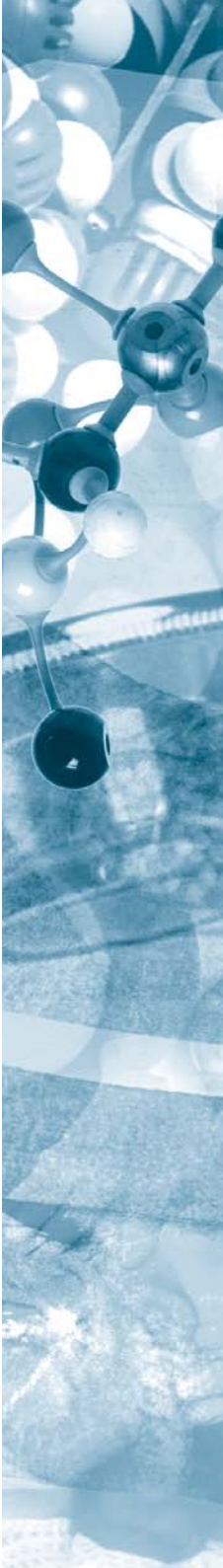
– Blocking Bag-1 protein expression *in vivo* by applying antisense oligonucleotides: To determine whether Bag-1 is functionally significant for the survival of regulatory T cells, antisense oligonucleotides were created complementary to the rat Bag-1 sequence. This was first analysed *in vitro* during mixed lymphocyte reaction (MLR). Flow cytometry analyses of oligonucleotide-treated MLR demonstrated decreased Bag-1 expression at day 5 in anti-CD4 mAb-treated cultures using 5 µM antisense oligonucleotides compared with control oligonucleotides. Bag-1 expression was reduced to 50% of control oligonucleotide-treated cultures. Moreover inhibition of Bag-1 expression leads to increased sensibility against both spontaneous and activation-induced apoptosis of the cells derived from anti-CD4 mAb-treated culture. 43.01% of the antisense oligonucleotide-treated MLR underwent AICD compared with 26.79% control oligonucleotide-treated MLR, respectively. Currently we are trying to inhibit Bag-1 expression in tolerance mediating T cells from anti-CD4 mAb-treated kidney recipients before adoptive transfer. That may show whether an enhanced Bag-1 expression is needed for the induction and maintenance of transplantation tolerance.

– Generation of Bag-1 knock-out mice: We have been able to isolate a genomic clone of the mouse Bag-1 sequence, which is currently being sequenced.

– Expression studies of other apoptosis-associated proteins (e.g. Bcl-2) *in vitro* and *in vivo*: Restimulation of previously activated T cells results in an increase of FasL expression. Interaction of FasL with its receptor Fas on the T cell surface initiates the apoptosis of the cells. This process, called activation-induced cell death (AICD), is very important for termination of immune responses. To investigate whether the relative resistance against AICD of anti-CD4 mAb-treated cultures is due to diminished FasL expression we analysed the FasL mRNA expression at different time points (0, 2, 9 and 18 hours) of AICD when restimulating the MLR cultures at day 5 with plate-bound anti-CD3 mAb. Restimulation of T cells results in a similar increase of FasL mRNA expression during the first two hours in both non-treated and anti-CD4 mAb-treated allogeneic MLR. Thus the enhanced protection against AICD of anti-CD4 mAb-treated cultures is not due to diminished FasL expression. In certain reports it was shown that resistance against Fas-mediated apoptosis is associated with a higher expression of the antiapoptotic protein Bcl-xl and with diminished expression of the proapoptotic proteins Bax and Bad. Flow cytometry analyses performed at day 5 after MLR revealed, in contrast to Bag-1, no differences of Bcl-xl and Bax expression between anti-CD4 mAb-treated and non-treated allogeneic cultures.

2. Characterisation of other genes differentially expressed in the renal transplantation model: We have further studied the cDNA fragment ACD4-2 that shows strong homology to the phosphatidyl-inositol-transfer protein (PITP). We generated a primer/probe panel for use in real time TaqMan® PCR. This panel will be used to analyse the expression of PITP during mixed lymphocyte reaction comparing anti-CD4 mAb-treated and non-treated allogeneic cultures. Furthermore, it will be used to investigate its expression at different time points in several transplantation models (see also point 3).

3. Expression studies in other transplantation models: So far we recruited samples from several transplantation tolerance models which will be used to analyse the expression kinetics of all isolated cDNA fragments.
First we used the non-depleting anti-CD4 mAb RIB5/2 (10x20 mg/kg body weight) to induce donor-specific tolerance against renal allografts in two different strain combinations: Wistar Furth to BDIX and DA to Lewis. The expression of the isolated cDNA fragments will be analysed at day 2, 5, 10, 14, 25 and >100 and compared to the expression at day 2 and 5 of non-treated control animals. The same expression studies will be done using heart allografts.
Furthermore, the expression of the isolated cDNA fragments will be analysed after CTLA-4-Ig treatment in renal and heart allografts.
The "adoptive tolerance" model was established by Tullius et al. A Fisher allograft is transplanted into Lew recipients leaving one of its own kidneys inside. The allograft undergoes chronic rejection. Four weeks later both kidneys will be removed and a second allograft is transplanted. This second renal allograft will be permanently accepted without any treatment. Samples from both the first allograft (as control) and the second allograft 2, 4, 8, 12 and 16 weeks after transplantation will be analysed for expression of the isolated cDNA fragments.
We generated primer panels for all isolated cDNA fragments that will be used to quantify its expression using the Sybrgreen application of PerkinElmer TaqMan® Cycler.
For the next year we would like to obtain the whole sequence of the genomic clone of mouse Bag-1 which allows us to start generating a knock-out mouse. Furthermore, we will analyse the expression kinetics of all of the isolated cDNA fragments in the above-mentioned transplantation tolerance models. At the moment we are recruiting samples from two different mouse tolerance models which will be used to compare the expression kinetics between different species. We continue characterising the function of the cDNA fragments ACD4-3 and ALLO-5.



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