Workshop Report:

Workshop Executive Summary and Report:

Imaging the Pancreatic Beta Cell

April 21-22, 2003
Bethesda, MD

National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK)
National Institute of Biomedical Imaging and Bioengineering (NIBIB)
Juvenile Diabetes Research Foundation International (JDRFI)

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INTRODUCTION

Why image the pancreatic beta cell?

Diabetes is a devastating disease that affects 16 million Americans. Type 1 diabetes results from the immune destruction of the pancreatic beta cell and loss of its secreted product, insulin. Type 2 diabetes results in an insulin resistant individual when the beta cells are no longer able to produce enough insulin to overcome this reduced response in the tissues. Diabetes can be treated with insulin or with drugs that either increase insulin secretion or tissue insulin sensitivity. Still, these patients are at very high risk for cardiovascular, retinal, kidney and neural complications due to elevated blood glucose, as well as dangerous hypoglycemic events. Our goal is to prevent or cure these diseases. This requires an increased ability to identify people before they become ill, new ways to monitor therapy, and a greater understanding of the pathogenesis of diabetes.

Recent advances in noninvasive imaging techniques such as Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) and other nuclear imaging techniques, and optical absorption or fluorescence spectroscopy and imaging, make it likely that a clinical exam to monitor beta cell number, mass, function, or lymphocyte infiltration can be established. This would allow at-risk individuals to be monitored prior to onset of diabetes. Patients could be monitored over the course of their disease; we could follow individual responses to drug therapy, and assess success of engraftment following islet transplantation. Researchers would learn about the natural history of diabetes. Persistant Hyperinsulinemia and Hypoglycemia of Infancy (PHHI) in neonates is another devastating disease where beta cell imaging could revolutionize treatment. These patients overproduce insulin and must often undergo partial surgical pancreatectomy. Imaging might be used to localize a focal or diffuse lesion to guide the surgeon and prevent removal of healthy tissue.

The National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), and the Juvenile Diabetes Research Foundation International (JDRFI) are pleased to sponsor the workshop 'Imaging the Pancreatic Beta Cell'. The goals for this meeting are to report ongoing work in beta cell imaging using MRI, PET, nuclear or optical technologies, form community among diabetologists and imaging researchers, and help NIDDK, NIBIB and JDRFI identify obstacles and opportunities toward a clinical exam for the measurement of pancreatic beta cell mass, number, function, inflammation, or engraftment.

EXECUTIVE SUMMARY

The workshop “Imaging the Pancreatic Beta Cell” showcased 22 speakers and16 posters. It was divided into three keynote lectures and sessions on Beta Cell Biology and Imaging, a lunchtime presentation on cellular imaging in the beta cell, and twelve 15-minute research presentations. Abstracts for these presentations are included in this report.

Greetings from the chairman, Dr. Susan Bonner-Weir, and the sponsoring organizations represented by Dr. Robert Nerem and Dr. Griffin Rodgers, were followed by a short introductory talk by Dr. Maren Laughlin that addressed the question, “Why do we need beta cell imaging? (discussed in the Introduction of this report).

The first keynote lecture by Dr. Kenneth Polonsky set the stage for the workshop. Dr. Polonsky described the pancreatic islet, and discussed clinical issues of type 1 and type 2 diabetes as well as research findings in animal models of diabetes that illustrated the need for the ability to image beta cell mass and function. Dr. Brian D. Ross (University of Michigan) delivered a talk on the state of the art of molecular imaging, outlining both the tremendous progress made to date and the challenges that remain, such as a need for contrast agents and instrumentation to increase detection sensitivity, and agents that can be targeted more specifically to the cells of interest in vivo. The final keynote address from Dr. David M. Harlan discussed the success and challenges in pancreatic islet transplantation as a therapy for type 1 diabetes. The ability to image endogenous and transplanted islet mass and function is critical for understanding the pathology of type 1 diabetes, the process of islet engraftment, and for finding alternative islet replacement strategies.

In the Beta Cell Biology session, Dr. Susan Bonner-Weir spoke about pancreas and islet anatomy, physiology, development, and the changes in the beta cell mass, function, protein expression and morphology that occur with age, body weight, and disease states (obesity, insulin resistance, hyperglycemia). Much of what is known in this area was learned from animal models or upon autopsy in people. The ability to image various aspects of the beta cell in vivo would provide tremendous data regarding the natural history of diabetes and beta cell development and turnover. Dr. Christopher Newgard discussed the unique metabolism and molecular biology of the beta cell and beta cell models associated with nutrient-stimulated insulin secretion. He also outlined some unique qualities of these cells that can be exploited in imaging, as well as some novel strategies used in his laboratory such as isotopic analysis using NMR and mass spec to study metabolism, or panning of peptide libraries to obtain cell surface targeting molecules. The final two speakers in this session, Drs. Klaus Kaestner and Ole Madsen, reported on efforts to elucidate the beta cell transcriptome, identify surface proteins, and generate antibodies to these potential cell markers. These tools will then be available to the beta cell imaging community. These are projects based on the NIDDK-supported Endocrine Pancreas Consortium (http://www.cbil.upenn.edu/EPConDB/) which has generated over 100,000 ESTs from over 20 mouse and human pancreatic and islet libraries. This effort is directed toward the identification of novel transcripts and production of a custom microarray chip (http://www.cbil.upenn.edu/EPConDB/Chips/pancChip.shtml). An additional effort, the Beta Cell Biology Consortium (http://www.betacell.org/), is focused on the basic islet biology leading to beta cell replacement in diabetes.

A working lunch featured a presentation from Dr. David Piston on cellular imaging of the beta cell. He described studies of the location and regulation of the main glucose-sensing enzyme, glucokinase, and on the role of compartmentalized redox metabolism in glucose-stimulated insulin secretion, measured as NAD(P)H fluorescence. A session on imaging techniques

featured Dr. Chien Ho and Dr. Samuel Achilefu. Dr. Ho described the use of MRI to monitor superparamagnetic iron oxide-labeled immune cell accumulation during the rejection of transplanted organs. Simultaneously, Dr. Ho was able to monitor function of the transplanted heart or kidney. This was a nice demonstration of the flexibility of MRI to measure a range of parameters of interest to the beta cell and diabetes communities. Dr. Achilefu reported on a variety of molecular probes used for optical imaging studies, as well as strategies for improving the penetration and depth resolution of light imaging.

Twelve beta cell imaging research presentations used a range of technologies including magnetic resonance imaging or spectroscopy, positron emission spectroscopy, and fluorescence and bioluminescence optical imaging. These could be roughly divided into cell imaging studies aimed at elucidating the basic biology of cellular processes such as insulin secretion or ion channel activity, and molecular imaging studies aimed at the visualization of beta cell mass, inflammation, engraftment, or function in living animals. Some of these were aimed at monitoring endogenous beta cells and inflammatory cells, while others focused on imaging transplanted islets. A variety of strategies were presented.

Transgenic or gene therapy techniques were employed to introduce fluorescent or bioluminescent proteins into beta cells or immune cells. Beta cells and inflammation were then monitored in mice, either as the endogenous tissue or after transplantation into a non-transgenic host.

Molecular targeting strategies were used to visualize infiltration of the NOD mouse (a model of type 1 diabetes) pancreas by cross-linked superparamagnetic iron oxide (CLIO) -labeled $CD8^+$ T cells. PET targeting approaches included the use of fluorinated glyburide analogs to selectively bind the beta cell. Nonspecific accumulation of signal in liver must be overcome in order to produce a fully reliable molecular labeling strategy. A number of talks addressed tool development for molecular imaging studies. These included the synthesis of new lipophilic MRI T1 relaxation contrast agents based on lanthanides that can enter the cell and are trapped upon enzymatic deesterification. Another approach takes advantage of both the MRI saturation transfer contrast and cell targeting ability of a new class of peptide-associated lanthanide complexes. A search for human beta cell targets is being conducted using transcript profiling of exocrine and endocrine pancreatic tissues, in combination with antibody screening of phage expression libraries for cell specific probes to be used in beta cell imaging.

Functional imaging studies included the measurement of intracellular and mitochondrial redox states via NADH and cytochrome optical spectra. An innovative MRI microscopy method based on the fact that paramagnetic Mn^{2+} mimics Ca^{2+} was used to monitor Ca^{2+} metabolism in islets.

There were several interesting presentations using cellular imaging to investigate the cellular processes in the beta cell. The mechanics, and ATP and calcium dependence of secretory vesicle movement and membrane fusion were visualized in single cells by evanescent wave microscopy using fluorescent and bioluminescent tags. Apoptosis were studied using calcium-sensitive fluorescent proteins and a fluorescent artificial caspase-3 substrate.

The meeting concluded with a discussion period led by Dr. Joseph Frank, centered on the barriers and opportunities that exist in translating the current work to the patient, where beta cell imaging can be used to monitor therapy and further our understanding of the pathogenesis and natural history of type 1 and type 2 diabetes. It was suggested that imaging immune system activity in the pancreas was likely to be possible in patients within a reasonable time frame.

Research in other types of imaging would be aided by using larger animals that have a solid pancreas (such as primates) and by new imaging tools. Optical methods show great promise because of the flexibility and range of probes, but new ways must be found to image more deeply into tissues, potentially through the use of slightly invasive instrumentation such as endomicrosopes. Although a noninvasive 3-dimensional technique in general, MRI sensitivity might also be improved with microprobes that could be brought in closer contact with the tissue of interest. It was noted that PET had a lot of promise for imaging small cell populations due to its inherent high sensitivity, and there should be more research to develop cell-specific PET probes. Cell markers are needed to provide molecular imaging targets. There is a need to increase the intensity of imaging agents or the sensitivity of detection methods by 2-3 orders of magnitude across all imaging modalities. Moreover, we need to speed development of new delivery methods, such as ultrasonically burst microbubbles, to get contrast agents directly to the tissue and/or into the cell of interest, in order to avoid nonspecific uptake by liver, spleen or macrophages. It was clear during the discussion that to solve the problem of bringing beta cell imaging into the clinic, it is imperative that teams of researchers work together to provide all the expertise regarding imaging modalities, chemical synthesis, pancreas and islet cell biology, and diabetes.

RECOMMENDATIONS

External Advisory Committee

Abass Alavi, Department of Radiology, University of Pennsylvania
Susan Bonner-Weir, Joslin Diabetes Center, Harvard University
Clark Colton, Department of Chemical Engineering, MIT
Ioannis Constantinidis, Department of Medicine, University of Florida
Joseph Frank, Laboratory of Diagnostic Radiology, Clinical Center, NIH
Robert Goldstein, Director of Research, Juvenile Diabetes Research Foundation
David Harlan, Transplantation and Autoimmunity Branch, NIDDK, NIH
Chien Ho, Department of Biological Sciences, Carnegie Mellon University
Dixon Kaufman, Department of Surgery, Northwestern University
Christopher Newgard, Stedman Nutrition and Metabolism Center, Duke University
Dean Sherry, Department of Chemistry, University of Texas, Dallas

Summary

The workshop was effective in:

- providing an overview of recent advances in beta cell biology;
- summarizing new approaches for cellular and molecular imaging;
- disseminating results of research funded by NIDDK RFAs DK-99-18 and DK-02-002 “Imaging Pancreatic Beta Cell Mass, Function, Engraftment or Inflammation”;
- fostering interactions between beta cell biologists, imaging scientists, and engineers;
- pointing to future applications of imaging techniques in understanding the natural progression of diabetes and monitoring clinical treatments for diabetes.

The Committee posed the following questions concerning the role of imaging in beta cell research and the treatment of diabetes:

(1) Can we image the natural progression of diabetes in humans?
Can we follow the number and viability of endogenous beta cells over time?

(2) Can we image transplanted islets in mice, larger animals, and humans?
Can we follow the number and viability of transplanted islets over time?

(3) Can we image immune cell infiltration of pancreatic beta cells?

(4) Can “functional imaging” be used to measure flux through metabolic pathways in beta cells?
How is beta cell metabolism unique, and how is it coupled to insulin secretion?

(5) Can we use information obtained from images as “surrogate markers” for clinical trials?

(6) Can we extend molecular imaging studies from mice to clinical studies with humans?

Recommendations

In order to move toward the goals of imaging native beta cells and transplanted islets in patients, the committee strongly encourages interactions between beta cell biologists, imaging scientists and engineers. One potential funding mechanism for these interdisciplinary programs is the BRP (Bioengineering Research Partnership) program within BECON. Another suggestion aimed at expanding the community and fostering collaboration was to form a "beta cell imaging interest group" and hold annual meetings.

The Committee pointed out differences in the goals and methods for imaging endogenous and transplanted beta cells. For example, transplanted beta cells are easier to label using molecular biology approaches, and can be used to study the role of angiogenesis and neurogenesis in beta cell engraftment. For this reason, it was felt that NIH consider ways to promote these efforts separately.

The Committee felt that the following topics would be useful areas of beta cell research in the near future:

1) Development of specific markers and probes that could be used to image beta cells

   a) Use information from the Beta Cell Consortium to identify unique beta cell surface markers, and disseminate this data to the imaging community;

   b) Explore a variety of ways to produce useful ligands (antibodies, small peptides, small molecules);

   c) Use FDA approved "probes" that could be extended to human studies;

   d) Explore imageble metabolic-functional correlates in the beta cell, and find ways of imaging the beta cell pathology found in type 2 diabetes (lipid accumulation, IAPP fibril formation);

   e) Explore the potential of targeted nuclear medicine probes for PET.

2) Development of "minimally invasive" laparoscopic approaches using micro-imaging (e.g., MRI, ultrasound, optical).

3) Use of large animal models with a solid pancreas (primates) and more extensive sharing of existing animal models.

4) Find ways to speed FDA approval of new imaging agents.

5) For transplantation studies, it should be possible in the short term to image inflammation, and these studies should be emphasized. Functional studies of transplanted islets, such as probes to measure apoptosis and metabolic activity, are needed. New transgenic animal models are needed.

6) New ways to use imaging to provide surrogate markers for type 1 prevention trials are needed.

7) Incorporation of insulin-secreting cells within artificial matrices that can be implanted for treatment of diabetes and monitored using imaging.

ADVISORY COMMITTEE

Susan Bonner-Weir, Ph.D.
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Harvard Medical School

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Molecular Biophotonics Laboratory
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PLANNING COMMITTEE

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Anna Retzke
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AGENDA

Chair: Susan Bonner-Weir, Ph.D.

Monday, April 21, 2003

6:00 p.m. - 7:00 p.m.	**Registration/Posters and Light Refreshments**
7:00 p.m. - 7:20 p.m.	**Welcome** *Robert Nerem, Ph.D., Special Scientific Advisor* *NIBIB* *Griffin P. Rodgers, M.D., Deputy Director* *NIDDK* *Susan Bonner-Weir, Ph.D.* *Harvard Medical School*
7:20 p.m. - 7:30 p.m.	**Introduction** Why Do We Need Beta Cell Imaging? *Maren R. Laughlin, Ph.D.* *NIDDK*
7:30 p.m. - 8:10 p.m.	**Keynote Lecture** Diabetes and the Beta Cell: Overview and Current Directions *Kenneth S. Polonsky, M.D.* *Washington University School of Medicine*
8:10 p.m. - 8:50 p.m.	**Keynote Lecture** In Vivo Molecular Imaging: Overview and Current Directions *Brian D. Ross, Ph.D.* *University of Michigan Medical School*
8:50 p.m. - 9:20 p.m.	**Keynote Lecture** Autoimmune Destruction and Islet Transplantation *David M. Harlan, M.D.* *NIDDK*
9:20 p.m. - 10:00 p.m.	**Posters and Light Refreshments**

Tuesday, April 22, 2003

7:00 a.m. - 8:00 a.m. **Registration and Refreshments**

8:00 a.m. - 9:30 a.m. **Session I: Beta Cell Biology**
Chair: Dixon B. Kaufman, M.D., Ph.D.
Northwestern University

8:00 a.m. - 8:30 a.m. **Important Issues of Islet/Pancreas Anatomy and Physiology That May Affect Imaging of the Beta Cell**
Susan Bonner-Weir, Ph.D.
Harvard Medical School

8:30 a.m. - 9:00 a.m. **Metabolism and Molecular Biology of the Beta Cell**
Christopher B. Newgard, Ph.D.
Duke University Medical Center

9:00 a.m. - 9:15 a.m. **An Informatics Approach to New Beta Cell Surface Markers**
Klaus H. Kaestner, Ph.D., M.S.
University of Pennsylvania

9:15 a.m. - 9:30 a.m. **Antibodies to Beta Cell Surface Markers**
Ole D. Madsen, Ph.D.
Hagedorn Research Institute

9:30 a.m. - 10:00 a.m. **Break**

10:00 a.m. - 11:30 a.m. **Session II: Research Presentations**
Chair: Chien Ho, Ph.D.
Carnegie Mellon University

10:00 a.m. - 10:15 a.m. **In Vivo Imaging of Autoimmune Response in Type 1 Diabetes**
Anna Moore, Ph.D.
Harvard Medical School

10:15 a.m. - 10:30 a.m. **Imaging of Secretory Vesicle Dynamics in Pancreatic Beta Cells**
Guy A. Rutter, Ph.D.
University of Bristol

10:30 a.m. - 10:45 a.m. **2- and 3-D Imaging of Mitochondrial Metabolic States of the Mouse Pancreas and Liver**
Britton Chance, Ph.D., D.Sc.
University of Pennsylvania

10:45 a.m. - 11:00 a.m. **Prospects for Beta Cell Imaging With PET**
Abass Alavi, M.D.
University of Pennsylvania

11:00 a.m. - 11:15 a.m. **Monitoring Transplanted Fluorescent Islets In Vivo**
Simon Watkins
Children's Hospital of Pittsburgh/University of Pittsburgh School of Medicine

11:15 a.m. - 11:30 a.m. **Beta Cell Mass and Function Following Transplantation**
Per-Olof Berggren, Ph.D.
Karolinska Institute

11:30 a.m. - 1:15 a.m. **Posters and Working Lunch**

11:45 a.m. - 12:15 p.m. **Cellular Imaging of the Beta Cell**
David W. Piston, Ph.D.
Vanderbilt University

1:15 p.m. - 2:45 p.m. **Session II: Research Presentations *(continued)***

1:15 p.m. - 1:30 p.m. **In Vivo, Real-Time, Noninvasive Bioluminescent Imaging of Transplanted Islets in a Functional Murine Model**
Dixon B. Kaufman, M.D., Ph.D.
Northwestern University

1:30 p.m. - 1:45 p.m. **Magnetic Relaxation Reagents for Cell Labeling and Imaging Applications**
Wen-hong Li, Ph.D.
University of Texas Southwestern Medical Center, Dallas

1:45 p.m. - 2:00 p.m. **Human Islet Antigen Discovery**
Paul E. Harris, Ph.D.
College of Physicians and Surgeons of Columbia University

2:00 p.m. - 2:15 p.m. **Using Bioluminescence to Noninvasively Image and Assess Transplanted Islet Mass**
Alvin C. Powers, M.D.
Vanderbilt University

2:15 p.m. - 2:30 p.m. **The Design of Responsive MRI Agents for Detecting Beta Cell Function: Will They Be Sensitive Enough?**
A. Dean Sherry, Ph.D.
University of Texas, Dallas

2:30 p.m. - 2:45 p.m. **Imaging of Pancreatic Beta Cell Function by Mn^{2+}-Enhanced MRI**
Brian B. Roman, Ph.D.
University of Illinois, Chicago

2:45 p.m. - 3:15 p.m. **Break**

3:15 p.m. - 4:45 p.m. **Session III: Imaging**
Chair: Christopher B. Newgard, Ph.D.
Duke University Medical Center

3:15 p.m. - 4:00 p.m. **Molecular and Physiological Imaging Using MRI**
Chien Ho, Ph.D.
Carnegie Mellon University

4:00 p.m. - 4:45 p.m. **Design of Molecular Imaging Agents**
Samuel Achilefu, Ph.D.
Washington University School of Medicine

4:45 p.m. - 5:50 p.m. **Discussion**
Translational Challenges for Cellular and Molecular Imaging
Joseph Frank, M.D.
Warren Grant Magnuson Clinical Center, NIH

5:50 p.m. - 6:00 p.m. **Concluding Remarks**
Susan Bonner-Weir, Ph.D.
Harvard Medical School

6:00 p.m. **Adjournment**

Diabetes and the Beta Cell: Increased Islet Apoptosis in PDX-$1^{+/-}$ Mice

James D. Johnson, Noreen T. Ahmed, Dan S. Luciani, Zhiqiang Han, Hung Tran, Jun Fujita, Stanley Misler, Helena Edlund, and Kenneth S. Polonsky
Washington University School of Medicine

PDX-1 is a homeobox gene critical for pancreatic development. Mice lacking one allele of the PDX-1 gene, thereby demonstrating a 50% reduction in PDX-1 gene expression, have worsening glucose tolerance with age and reduced insulin release in response to glucose, KCl and arginine from the perfused pancreas. Surprisingly, insulin secretion in perifusion or static incubation experiments in response to glucose and a variety of other secretagogues was similar in islets isolated from PDX-$1^{+/-}$ mice compared to PDX-$1^{+/+}$ littermate controls. Glucose sensing and islet Ca^{2+} responses to glucose, glyceraldehyde, ketoisocaproic acid, KCl and carbachol were normal. Glucose, arginine and KCl generated Ca^{2+} signals in single cells from both PDX-$1^{+/+}$ and PDX-$1^{+/-}$ islets, although with distinct kinetics. Depolarization-evoked exocytosis and Ca^{2+} currents in single PDX-$1^{+/-}$ cells were not different from controls, arguing against a ubiquitous β -cell stimulus-secretion coupling defect. However, isolated PDX-$1^{+/-}$ islets and dispersed β-cells were significantly more susceptible to apoptosis at basal glucose concentrations than PDX-$1^{+/+}$ islets. Expression of the anti-apoptotic genes, Bcl_{XL} and Bcl-2, were reduced in PDX-$1^{+/-}$ islets. *In vivo*, increased apoptosis was associated with abnormal islet architecture, positive TUNEL, active caspase-3 and lymphocyte infiltration. Although similar in young mice, both β -cell mass and islet number failed to increase with age and were ~50% less than controls by 1 year. These results suggest that an increase in apoptosis, with abnormal regulation of islet number and β -cell mass, represents a key mechanism whereby partial PDX-1 deficiency leads to an organ-level defect in insulin secretion and diabetes.

Ross, Brian D.

In Vivo Molecular Imaging: Overview and Current Directions
Brian D. Ross
University of Michigan Medical School

Not available at press time

NIH Intramural Diabetes Initiatives Emphasize the Need for Non-invasive Islet Imaging

Klaus Pechhold, Kristina I. Rother, Benigno J. Digon III, Boaz Hirshberg, David M. Harlan
National Institute of Diabetes & Digestive & Kidney Diseases

A reliable, non-invasive technique for measuring beta cell mass in vivo is critically needed. Utilizing both animal models and clinical trials, investigators within the NIDDK's Transplantation & Autoimmunity Branch (TAB) are exploring mechanisms underlying T1DM pathogenesis, and testing means of preserving or enhancing functional beta cells mass. The basic features of several such studies will be described along with our current efforts to quantify functional islet mass. Brief but specific attention will be directed toward the following TAB projects:

1) A rat insulin promoter (RIP) transgenic mouse system for assessing T1DM immunopathogenesis. The model makes use of transgene-encoded beta cell CD80 expression which results in these mice displaying an extraordinary susceptibility towards beta cell-specific autoimmune attack by MHC class I restricted CD8+ T cells. Variants of this model have introduced defined "experimental" beta cell autoantigens like the lymphocytic choriomeningitis virus glycoprotein (LCMV-GP), and utilize other transgenic mice that express transgene encoded T cell receptors (TCR) specific for beta cell autoantigens in the proper context.
2) An assay system has been adapted from the experimental autoimmune diabetes model described in (1) to test the ability of adoptively transferred pluripotent stem cells (adult hematopoeitic, embryonic, or other) to give rise to pancreatic beta cells in vivo.
3) A rodent islet transplantation model exploring different islet implantation sites for potential eventual clinical application.
4) A non-human primate system for assessing beta cell mass prior to diabetes induction, for streptozotocin-induced and specific beta cell destruction, and for measuring islet function following islet cell transplantation. Functional measurements have been correlated with histological findings and will be reported.
5) A clinical protocol testing whether orally administered interferon-alpha can abrogate the beta cell loss underlying T1DM.
6) TAB clinical islet cell transplantation efforts and patient follow-up data.
7) An analysis of a significant subset of islet cell transplantation candidates excluded from the protocol due to persistent c-peptide production despite "classic" T1DM being diagnosed years previously.
8) New protocol(s) designed to assess whether beta cell function can be augmented by the administration of agent(s) predicted in animal models to promote islet neogenesis. Our analysis of factors important for such a study will be discussed.

Just as the "glucose hypothesis" could not be meaningfully supported by experimental data until the Diabetes Complications and Control Trial was made possible by advances in blood glucose monitoring and new insulin delivery systems, so too important advances directed toward the support or enhancement of beta cell mass will likely need to await technical advances allowing the accurate and non-invasive measurement of that mass.

Important Issues of Islet/Pancreas Anatomy and Physiology that May Affect Imaging of the β cell

Susan Bonner-Weir
Joslin Diabetes Center, Harvard Medical School

Diabetes results when there is an inadequate β cell mass whether by destruction (type 1) or inadequate compensation for increased functional need (type 2 and MODY). Non-invasive assessment of β cell mass would provide an important tool for both therapeutic interventions and better understanding of the natural history of diabetes, both type 1 and type 2. The anatomy of the islets of Langerhans and their location within the parenchyma of the pancreas must be considered in designing strategies for this imaging assessment. Some of the issues that need consideration are:

- β cells are the main component (about 70% of the cells) of the islets of Langerhans, but their proportion changes with age.
- The islets themselves are highly vascularized microorgans that are composed of 4 endocrine cell types in a non-random organization and are scattered throughout a sea of acinar tissue of the pancreas. Islets are only about 1-2% of the pancreas and are not randomly distributed throughout the pancreas. They may be as small as 20 μm diameter or as large as 400 μm diameter.
- In rodents this pancreas is diffuse and difficult to immobilize in one plane for imaging.
- The β cell mass is dynamic with compensatory changes (both expansion and involution) to maintain glucose homeostasis. These changes can be in number or volume of cells with both the increasing size of islets by replication or cell hypertrophy and increasing numbers of islets by neogenesis from precursors/progenitors. In both rodents and human, the β cell mass is linearly related to body weight/BMI.
- β cells have a common origin with the other islet endocrine cells, the acinar cells and the pancreatic ductal cells. So many potential markers of β cells may also be expressed in these other pancreatic tissues.
- Many characteristics of β cells change with exposure to chronic hyperglycemia. With active secretion, granule membrane molecules may be inserted into the plasma membrane to a greater degree. The cells may become degranulated but may still be functional. Cell surface markers such as GLUT2 and GLP-1 receptor are down regulated with hyperglycemia.

Newgard, Christopher B.

Metabolism and Molecular Biology of the Beta-Cell

Christopher B. Newgard
Duke University Medical Center

Insulin secretion is absent in type 1 diabetes and impaired in type 2 diabetes. Technologies that might allow visualization of the process of autoimmune destruction of beta-cells in type 1 diabetes and changes in beta-cell mass and function during development of type 2 diabetes would be of tremendous value for better understanding of disease pathogenesis and development of new therapeutic strategies. This lecture will focus on providing a contextual background for the development of targeting methods for islet beta-cells, including a description of our laboratory's inter-disciplinary approach for the study of mechanisms of fuel-regulated insulin secretion and beta-cell survivial. Tools that will be described include; 1) novel cell line models for studying insulin secretion and beta-cell survival; 2) gene discovery and genetic engineering technologies; 3) biophysical methods such as NMR and mass spectroscopy for "metabolic profiling" of beta cells (in collaboration with A.D. Sherry and colleagues); and 4) identification of peptides capable of specific targeting to beta-cells (in collaboration with S.A. Johnston, K. Brown, and colleagues).

Kaestner, Klaus H.

An Informatics Approach to New Beta Cell Surface Markers

Angel Pizzaro, Chris Stoeckert and Klaus H. Kaestner
University of Pennsylvania

The Endocrine Pancreas Consortium was formed in late 1999 to derive and sequence cDNA libraries enriched for rare transcripts expressed in the mammalian endocrine pancreas. Over the past three years, the Consortium has generated twenty cDNA libraries from mouse and human pancreatic tissues and deposited more than 150,000 sequences into the public EST databases. Sequence analysis showed that these clones cluster into 9,464 assembly groups (approximating unique transcripts) for the mouse, and 13,910 for the human sequences. Of these, more than 4,300 were unique to Consortium libraries. We have now taken a computational approach to identify genes encoding potential cell surface antigens as a first step towards the generation of antibodies for sorting of beta-cells and their precursors. The following four criteria were used: presence of a signal peptide, presence of at least one transmembrane domain, presence of the word “membrane” in the clone description, and identification of the gene in the Skarnes gene trap lines. Of the 9,464 mouse assembly groups, 779 clones met at least two of these criteria. For 327 of these 779 genes full-length cDNA clones are available. In a final step, these 327 genes were screened manually to exclude those that encode proteins that are secreted or present on intracellular membranes like Golgi or mitochondria. The open reading frames of the remaining 192 clones will be transferred into the Creator shuttle vector and resequenced. The final clones will be transferred into a GST plasmid by Cre-mediated recombination and the GST-fusion proteins purified and shipped to Dr. Madsen’s laboratory for antibody production.

Madsen, Ole D.

Antibodies to Beta Cell Surface Markers

Ole D. Madsen
Hagedorn Research Institute, Gentofte, Denmark

The Antibody Core of the NIDDK-funded BCBC (Beta Cell Biology Consortium) has been given the mandate to produce and distribute antibodies of interest to the field of pancreas and beta cell developmental biology. Antibodies recognizing particular transcription factors as well as other nuclear/cytoplasmic proteins are unique histological markers for the study of pancreatic beta cell ontogeny. Much progress has been made in elucidating and understanding sequential events leading to endocrine specification and maturation. However, with the wish of moving from pancreas development into in vitro stem cell biology it will become mandatory to develop antibodies to surface markers. Strategies taken to achieve some of these goals include future construction of tagged mice, so-called rainbow mice that may allow for the isolation of uniquely staged cells, which followed by gene-chip analyses may facilitate the identification of unique cell surface epitopes to be used for subsequent antibody formation. Such epitopes might be selected for optimal conservation between muse and man – and thus provide tools to monitor human beta cell maturation ex vivo. Techniques used include conventional immunization for polyclonal antibodies, hybridoma derived monoclonal antibodies and phage-display techniques. The latter technique is additionally useful to possibly improve binding affinities for desired monoclonal antibodies. It is our hope that such future antibodies might find use also for imaging purposes of various pancreatic cell subpopulations.

Moore, Anna

In Vivo Imaging of Autoimmune Response in Type 1 Diabetes

Anna Moore
Massachusetts General Hospital, Harvard Medical School

MHC class I-restricted CD8+ T cells play important roles both early, in the initiation of Type I diabetes, and later on, as effectors of beta–cell damage. The studies on pancreatic tissues are generally limited to autopsy, and methods for non-invasive monitoring of T cell accumulation currently do not exist. In vivo imaging of T cell invasion in real time would have significant impact on managing clinical diabetes, understanding its pathogenesis, and pancreas and/or islets transplantation. Based on our prior experience in cell labeling with superparamagnetic iron oxide nanoparticles we synthesized a novel MR imaging probe consisted of biotinylated complex of H-2Kd class I molecule with antigenic peptide (NRP-V7) conjugated to nanoparticles through avidin linkage. This probe was recognized by a large fraction of diabetogenic CD8+ T cells in the NOD mouse expressing a transgenic, highly diabetogenic, NRP-V7-reactive T cell receptor but was not recognized by CD8+ T cells from healthy NOD mice. Since it is clinically relevant to be able to administer the probe systemically, we also studied direct labeling of diabetogenic T cells in vivo. Furthermore, in vivo MR imaging experiments showed accumulation of labeled diabetogenic CD8+ T cells in mouse pancreas after adoptive transfer over time. For 16 days we observed the decrease of signal intensity in the pancreas, which indicates the accumulation of the labeled cells. We demonstrated that the progression of diabetes could be observed in vivo by MR imaging, a technique that may prove useful in early detection and monitoring of the disease non-invasively after therapeutic intervention and/or transplantation.

Rutter, Guy A.

Imaging of Secretory Vesicle Dynamics in Pancreatic Beta-cells

Guy A. Rutter
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Objectives. To monitor the movement of insulin-containing dense core secretory vesicles in real time in single living pancreatic islet beta-cells, using digital imaging, and to explore the underlying mechanisms.

Methods and Results. We have developed a number of targeted, green fluorescent protein- and aequorin-based probes to monitor vesicle behaviour and Ca^{2+} ion fluxes. These tools have allowed us to demonstrate that nutrients including glucose stimulate insulin release from secretory vesicles in part by activating their recruitment from a reserve pool, located several granule diameters from the plasma membrane. Evanescent wave microscopy reveals that exocytosis then occurs in insulin secreting cells as both full fusion and "kiss and run" events [1]. Kinesin I heavy chains are essential to drive vesicle translocation, since microinjection of cDNA encoding dominant-negative (KHCmut) KHC motor domain blocks all vesicular movements. Correspondingly, expression of KHCmut strongly inhibits the sustained, but not the acute, stimulation of secretion by glucose [2]. By contrast, vesicle behaviour and exocytosis are unaffected by disruption of cytoplasmic dynein through overexpression of p50-dynamitin. Overexpression of AMP-activated protein kinase also inhibited vesicle movement and insulin release implicating the latter enzyme in the glucose sensing machinery of beta-cells [3].

Using vesicle-targeted Ca^{2+} probes (VAMP2-aequorin, phogrin.cameoleon), and RNA silencing, we have also explored the role of intravesicular Ca^{2+} ions in regulating the exocytotic process. RNA silencing suggests that vesicles accumulate Ca^{2+} via ATP-dependent Ca^{2+} pumps, distinct from sarco(endo)plasmic reticulum Ca^{2+}-ATPases and including the mammalian orthologue of yeast plasma membrane related 1 (PMR1). Receptors for both ryanodine (Type I) and nicotinic acid adenine dinucleotide phosphate (NAADP), located on the vesicle membrane, appear to allow highly localized release of Ca^{2+} at the vesicle surface, which may contribute to both vesicle movement and fusion at the plasma membrane [4]. Future efforts will aim to determine whether any association may exist between mutations in the genes encoding these proteins and type 2 diabetes mellitus.

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Chance, Britton

2- and 3-D Imaging of Mitochondrial Metabolic States of the Mouse Pancreas and Liver

Britton Chance, Zhihong Zhang, Gang Zheng, and Jerry Glickson
University of Pennsylvania

The complexity of metabolic pathways explored by various tracer and optical imaging techniques emphasizes the need to look at the end result of such activations upon the mitochondrial metabolic state. The fluorescence of the matrix space NADH and the flavoprotein prosthetic groups of the pyruvate and ketoglutarate dehydrogenase forms a redox couple that has been a key to in vivo studies of brain, liver, and more recently pancreas biochemical assays of NADH and NADPH, yielding excellent agreement between the fluorescent studies and the micro analytical biochemistry and cardiac muscle studies validating that snap freezing and the redox state could be preserved with high fidelity. The snap freeze technique has several advantages: 1) prolonging the instrument measurement time and hence increases of the signal to noise ratio; 2) causing nearly 10 fold increase of fluorescence quantum yield due to slower dark combination reactions at (77 K); 3) allowing low excitation intensities with no photobleaching; 4) allow biochemical and histopathological verification of optical studies; 5) metabolic heterogeneity is displayed by 4x104 point histograms; and 6) precision imaging in 2 and 3D with 40μ resolution

Fluorescence and reflectance signals are obtained for NADH, flavoprotein, HbO_2 + Hb, HbO_2 and for a variety of fluorochromes which includes GFP, various forms of fluorescent glucose and affinity labels for somatostatin, LDL, folate, cathepsin, etc, currently focused on cancer signatures but can be synthesized for particular genetic expressions of the pancreatic islets.

Specific metabolic activation of the mitochondrial redox state of the beta cells is monitored by pyrodeoxyglucose 6 phosphate, etc. Observations are made of the change of the ratio of flavoprotein to NADH fluorescence and the accumulation of the phosphorylated fluorescent glucose. 2- and 3-dimensional images are compared These measurements lay the foundation for a rational approach to the mitochondrial function and the delivery of reducing power ATP generation and Ca uptake. Performance of the imager in creating a 3-D fluorescence images of redox state and glucose uptake. A close proportionality of the accumulation of fluorescent glucose and the changes of the mitochondrial redox state is observed.

Alavi, Abass

Prospects for ß-cell Imaging with PET

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Diabetes mellitus is characterized by a deficiency in insulin secretion and ß-cell loss. A non-invasive method to monitor the status of ß -cells during the silent phase of prediabetes or after pancreas transplantation will be useful for the treatment of diabetes mellitus. We have synthesized a series of fluorinated glyburide analogs [2-fluoroethoxy (**3**), 2-fluoropropoxy (**4**), 2-fluorobutoxy (**5**), 2-*p*-fluorobenzyl-5-chloro-glyburide (**6**) and their 5-iodo analogs (**7-10**)], and study their structure-activity relationships as insulin secretagogues and ß -cell imaging agents. The results showed that compounds **3-10** all stimulate insulin release from rat islets with compounds **6** and **10** being the highest. The *in vitro* binding affinities of F-18 labeled **3-5** and **7-9** to whole ß -cells (TC3 and Min 6) showed that up to 2% of F-18 radioactivity was bound to ß -cells and that the binding of compounds **4, 5, 8, 9** to ß -cells was less saturable compared to compounds **3** and **7**. In conclusion**,** this study shows that increasing the length of the side chain of the fluorinated glyburide analogs resulted in an increase of their non-specific binding to whole ß -cells and that F-18 labeled compounds **3, 7** and **6, 10** may serve as ß -cell imaging agents.

Monitoring Transplanted Fluorescent Islets In Vivo

Simon Watkins, Peter Drain, and Massimo Trucco
Children's Hospital of Pittsburgh and University of Pittsburgh School of Medicine

Our goal is to combine pancreatic islet allotransplantation and regenerate islet cell-specific tolerance in diabetic animals. Such protocols will allow these animals to be cured of diabetes without the need for immunosuppression. Once successfully tested in human trials, these combined procedures can be used in young diabetic patients. Non-invasive live-cell fluorescent monitoring of donor islets and host immune cells will dramatically facilitate the development of successful protocols. Optical imaging tools have been transformed from primarily descriptive methods to an array of methodologies to visualize and quantify behaviors of molecules, cells, and organs in 3D space and time. The molecular reporters and multi-photon and multi-mode live-cell imaging continue to undergo breathtaking technological developments. Our first major advance was the development of Ins-C-GFP, a live-cell fluorescent reporter of insulin content and secretion. For Ins-C-GFP, the emerald variant of GFP was placed in-frame within the C peptide connecting the A and B chains of murine proinsulin II. The Ins-C-GFP reporter construct was inserted into the E1 region of an E1, E3 deleted adenoviral vector and expressed in wild-type mouse islets by infection. Ins-C-GFP expression gave rise to beta cells containing extraordinarily intense punctate fuorescence, as compared to controls [1, 2]. Our second advance was Ins-C-TIMER: (1) "TIMER" is a derivative of dsRed1 that changes from green fluorescence to red fluorescence, effectively timing the first 24 hrs of the life-time of the protein. We generated Ins-C-TIMER transgenic animals, which demonstrated beta cell can be easily monitored in whole, live animals. (2) We observed C-TIMER through a standard coverslip "porthole' sutured to peer through the body wall of mice. Portholes in NOD mice transplanted, under the kidney capsule, with transgenic Ins-C-TIMER- labeled islets, will be used to monitor insulin content, secretory competence, and cell death over the life-time of islet transplants. (3) We have used fluorescent protein labeling of macrophages and lymphocytes to simultaneously monitor immune reactions to the transplanted C-TIMER islets. (4) We plan to use these imaging advances to quantify immune responses to transplanted islets that have been ex-vivo transfected with immunoregulatory genes, possibly able to protect the islets from recurrence of autoimmunity and allorejection. (5) Newly generated $InsII^{TIMER}$ -$InsI^{-}$- *InsII* – mice will allow to perform studies similar to the ones described before, having, however, all the synthesized proinsulin TIMER-tagged and insulin and C-TIMER synthesized in a 1:1 stoichiometric ratio. The construct necessary to knock-in C-TIMER into the endogenous murine *InsII* locus, has been prepared, sequenced, and transfected into ES cells. ES clones were screened for the presence of the modified gene in the appropriate location and quantity. Mice with all synthesized proinsulin fluorescently labeled will be generated by crossing homozygous $InsII^{TIMER}$ knock-in mice with mice containing null alleles of *InsI* and *InsII*. These advances should not only facilitate identification of properties of donor islet survival and function, together with host responses that correlate with successful conversion of host diabetic NOD mice to euglycemia but also advance understanding of what will be required to cure diabetes in children.

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Berggren, Per-Olof

Beta Cell Mass and Function Following Transplantation

Per-Olof Berggren
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Not available at press time

Piston, David W.

Cellular Imaging of the Beta Cell

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The convergence of newly developed instrumentation and optical probes allows us to examine quantitatively dynamic processes within ever more complicated biological systems. These new tools are affecting how almost all biological optical microscopy experiments are done. Studies of living cells, for instance, have dramatically changed since the introduction of the Green Fluorescent Protein (GFP). By using various quantitative fluorescence imaging methods of multi-colored GFPs fused to the glucose sensing enzyme glucokinase (GK), we discovered that the location and activity of beta cell GK is acutely regulated by glucose. Since small changes in beta cell GK activity have large effects on insulin secretion, this regulation plays a significant role in the control of blood glucose concentration. Using fluorescence recovery after photobleaching (FRAP) of GK-GFP bound to insulin granules, we found that glucose stimulation increased their mobility, thus indicating that GK was released into the cytoplasm. Use of a GK fusion protein labeled with two different GFP variants revealed changes in fluorescence resonance energy transfer (FRET) ratios that correlated with increased GK activity in the cytoplasm. Using pharmacologic inhibitors of insulin secretion and insulin receptor signaling, we found that glucose-induced changes in FRAP and FRET required autostimulation of beta cells by secreted insulin. These findings provide a mechanism whereby the glucose sensing ability of the beta cell is tightly coupled to insulin signaling.

As molecular imaging methods have been perfected in single cells, we have also applied them to imaging of tissues and whole organisms. We have measured pancreatic β-cell metabolism during glucose and pyruvate stimulation of pancreatic islets by quantitative two-photon NAD(P)H imaging. We have developed methods to delineate the NAD(P)H signals from the cytoplasm and mitochondria, and show that the metabolic response of these two compartments are differentially stimulated by glucose and other metabolites. Glucose causes sustained NAD(P)H increases in both compartments, and these increases result in insulin secretion. Interestingly, the mitochondrial NAD(P)H increase is delayed with respect to the cytoplasmic change by ~20 seconds, indicating that the mitochondrial change depends on the smaller, but more rapid, cytoplasmic increase. In contrast, pyruvate-stimulated NAD(P)H increases are well below those induced by glucose, and do not cause insulin secretion. Low pyruvate concentrations decrease cytoplasmic NAD(P)H without affecting mitochondrial NAD(P)H, while higher concentrations increase both cytoplasmic and mitochondrial levels. However, this mitochondrial increase is transient and equilibrates to a level just above baseline. Inhibitors of the mitochondrial pyruvate-transporter and/or the malate-aspartate shuttle can be utilized to resolve the mechanisms behind the glucose- and pyruvate-stimulated NAD(P)H responses. Glucose-stimulated mitochondrial-NAD(P)H and insulin secretion are independent of mitochondrial metabolism, but dependent on NAD(P)H shuttling. These inhibitors enhance the pyruvate-stimulated cytoplasmic NAD(P)H response and surprisingly the malate-aspartate shuttle inhibitor enables pyruvate-stimulated insulin secretion. This data suggests a primary role for glycolysis in glucose-stimulated insulin secretion.

In Vivo, Real-time, Non-invasive Bioluminescent Imaging of Transplanted Islets in a Functional Murine Model

Dixon B. Kaufman, Marshall S. Baker, Jeffrey J. Nelson, Xiaomin Zhang, and Xiaojuan Chen
Feinberg School of Medicine, Northwestern University

Early loss of transplant islet mass at the implantation site significantly reduces the functional potential of the graft. The availability of a technical method to serially non-invasively quantify and monitor islet graft viability would be an important step in improving outcomes. Toward that end, we have developed a model of islet imaging that can be used to assess the viability of a small mass of transplanted islets in the living mouse post-transplant in real-time. A transgenic mouse strain, referred to as the RIP-luc mouse (FVB/N strain, $H\text{-}2^q$) uses the rat insulin gene II promoter to drive luciferase expression taken from the pGL3 vector. The RIP promoter activates the luciferase gene in the pancreatic islets. Recent technical advances have made it possible to image weak visible light sources in small animals using charged coupled device (CCD) cameras with enhanced signal-to-noise ratios and low background signal. Transplanted RIP-luc islets (>200 islets/recipient) corrected the hyperglycemic state. Intra-peritoneal luciferin injection resulted in islet bioluminescence that peaked at 10-15 minutes. The sensitivity of the optic imaging system was tested by implanting a variable number of islets (50, 200 and 400/recipient) from the RIP-luc donor strain to the renal subcapsular space of isogeneic recipients. Digital photon counting image computer analysis demonstrated that in vivo bioluminescence intensity was proportional to the transplanted islet mass. Importantly, bioluminescent intensity was detectable with as few as 50 islets/recipient. Finally, isogeneic RIP-luc strain islets (200/recipient) were implanted intraportally to the liver and intraperitoneal on a gel maxtrix structure. Bioluminescence was localized to the site of implantation. These pilot studies indicated the feasibility of real-time non-invasive post-transplant bioimaging that will be useful to define how the fate of the viable islet mass is affected by anatomic and immunologic considerations that directly relate to transplant functional outcome. This technique should be useful in assessing interventions to sustain or increase islet mass following transplantation.

Li, Wen-hong

Magnetic Relaxation Reagents For Cell Labeling and Imaging Applications
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Beta-cells of the islets of Langerhans play important roles in regulating metabolism through the action of secreting insulin. Injury of ß-cells occurs in diabetes. The details of this injury process are poorly understood, in part because there is no method to localize ß-cells in vivo. Attempts to treat diabetes by introducing ß-cells are also limited by uncertainty about the fate of the cells.

Magnetic resonance imaging (MRI) is a truly non-invasive imaging technique that provides 3-D images of internal structures of living organisms. The capability of being able to probe opaque specimen at high spatial resolutions makes MRI an attractive choice for imaging islet β-cells whose mass is only about 1% of the pancreas.

We are developing MRI contrast agents for labeling and imaging beta-cells in vivo. We have designed and synthesized a novel class of lanthanide complexes for cellular imaging applications. These lipophilic T1-relaxation reagents can be taken up by fully intact cell populations to high concentrations. Mammalian cells labeled with these agents showed dramatic enhancement in image intensity over control cells using T1-weighting. In addition, labeled β-cells grew and secreted insulin normally in response to glucose stimulations. Currently we are applying these new relaxation reagents to track implanted β-cells in animal models. This part of work may provide us a non-invasive imaging method for future studies of transplanted islets or insulin secreted ß-cells in encapsulated devices for treating diabetes.

Harris, Paul E.

Human Islet Antigen Discovery

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New York Regional Islet Resource Center, College of Physicans and Surgeons of Columbia University in the City of New York

The overall goal of these studies is to develop reagents suitable for imaging human islets in the context of islet transplantation or Type 1 Diabetes management. Using antigen mining techniques borrowed from tumor immunology, combined broad scale gene expression analysis and bioinformatics, we propose to characterize the cell surface of islet tissue to identify candidate markers that can distinguish islets from the surrounding ductal, vascular, exocrine and liver tissue. To date we have used transcript profiling to obtain maps of the genes expressed by human islet tissue, exocrine pancreas, whole pancreas and liver tissue. This information has allowed us to generate a list of candidate molecules that are expressed by human islets but not by the exocrine component of the pancreas nor by the liver. Using bioinformatics and data in public repositories, expression of candidate markers in other tissue can be checked, allowing us to eliminate genes coexpressed by islet tissue and other tissues not directly analyzed by us. We are currently validating the tissue specificity and distribution of candidate markers using PCR in libraries of cDNAs prepared from a variety of tissues. Candidate markers include several transmembrane receptors (olfactory and cholinergic receptor family members) with limited tissue distributions (e.g. Islets, CNS and ovarian epithelium). In Parallel, with these studies we are using an adaptation of the SEREX technique (a.k.a. antibody screening of phage expression libraries) to identify islet cell surface associated molecules that may be useful in imaging. Both xenogeneic and IDDM patient antiseras will be used for library screening.

Using Bioluminescence to Non-invasively Image and Assess Transplanted Islet Mass

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To assess the mass of transplanted pancreatic islets, we are using in vivo bioluminescence imaging (IBI), a process whereby the emission of visible light generated by a luciferase-catalyzed reaction of molecular oxygen with luciferin, is detected from an external vantage point. Human islets (isolated at the NIH or from JDRF Human Islet Distribution Network) are infected in vitro with an adenovirus encoding luciferase and then transplanted into the liver (by infusion into the portal venous system) or beneath the renal capsule of NOD-SCID mice. NOD-SCID mice are immunodeficient, accept xenografts, allow long-term expression of genes introduced by adenoviral-mediated gene transfer, but do not develop diabetes or insulitis. Prior to transplantation, the bioluminescence of luciferase-expressing islets was proportional to islet mass and correlated with luciferase activity assessed with a luminometer. After transplantation, mice were imaged after an intraperitoneal injection of luciferin and bioluminescence of luciferase-expressing human islets was detected using a highly sensitive, liquid nitrogen-cooled CCD camera. Bioluminescence was seen in the expected anatomic distribution (liver or kidney) with a low background. Using quantitative image analysis, bioluminescence 3.5 weeks after transplantation was proportional to the original islet mass (500, 1000, or 2000 luciferase-expressing human islets). Bioluminescence could be detected after transplantation of as few as 100 transplanted human islet equivalents, correlated with glucose-stimulated human plasma insulin using a species-specific radioimmunoassay, and has been reassessed repeatedly for more than 4 months in the same animal. In vivo bioluminescence imaging may be useful in serial measurements of transplanted islet mass and in assessing interventions to sustain or increase islet mass following transplantation.

Sherry, A. Dean

The Design of Responsive MRI Agents for Detecting ß-cell Function: Will They Be Sensitive Enough?

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MR image contrast may be altered by paramagnetic or ferromagnetic contrast agents that shorten the T_1 or T_2 of tissue water. Most often used are Gd^{3+} complexes that have a single inner-sphere water molecule in rapid exchange with bulk water. The efficiency of Gd^{3+}-based agents is sensitive to several physical parameters, including the bound water lifetime (tM) and the rotational correlation time of the complex (t_R). Hence, a Gd^{3+}-complex attached to any protein or cell-targeting moiety can potentially form the basis of a protein-specific [1] or tissue-specific MR imaging agent. The limits of detection of Gd^{3+}-based agents, however, have not been fully delineated. An alternative method for introducing tissue contrast is to indirectly saturate a portion of the bulk water signal via pre-saturation of slowly exchanging OH or NH groups in endogenous or exogenous compounds [2]. This is referred to as chemical exchange saturation transfer (CEST). Very recently, a new class of paramagnetic Ln^{3+} complexes that exhibits slow water exchange has been used to introduce image contrast via the CEST mechanism [3]. Exchange theory predicts that this PARACEST effect has the potential of being substantially larger for introducing contrast than the relaxation effects offered by conventional Gd^{3+}-based T_1 agents. Thus, one could envision the design of PARACEST agents that not only target the ß-cell but also report the functional status of the cell.

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Roman, Brian B.

Imaging of Pancreatic Beta Cell Function by Mn^{2+}-Enhanced MRI

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University of Illinois at Chicago and Boston University

The pathologies resulting from Type I diabetes have serious morbidity and mortality consequences. To overcome the decrease in endogenous insulin production, research endeavors have focused on several alternatives for insulin supplementation including implantable biocapsules containing insulinoma cells, and transplantation of intact pancreatic islets. The usefulness of these approaches is usually assessed via measurement of serum glucose levels, which provides little direct information on the implant function. In light of clinical needs, it is critical to develop non-invasive techniques that can ascertain the implant efficacy, assess functional status and viability of these cells. However, available methods to non-invasively assess the function of these implants and transplants are limited. MRI techniques currently implemented to study the pancreas are sensitive to lesions and tumors at high spatial resolution but do not offer much functional information.

It would be useful if MRI techniques could be developed that would enable imaging of a parameter directly related to pancreatic, i.e. ß-cell, function. Glucose stimulated influx of calcium into ß-cells is necessary for insulin release. Divalent manganese ions (Mn^{2+}) can enter cells through voltage-gated calcium channels, are paramagnetic and Mn^{2+} accumulation in ß-cells should alter relaxation times offering an approach to sensitize MRI to calcium influx in these cells. This technique may be extremely useful in the evaluation of novel therapies being developed for physiologically regulated insulin delivery.

Our group is developing new approaches to cellular delivery based on micro- and nanotechnology. The ß-cell functional imaging being developed will be used to evaluate micromachined nanoporous biocapsules that are specifically engineered to provide an immunoisolative and functional environment for insulin-secreting cells. Additionally this technique is being implemented to asses function of isolated pancreatic islets which is also being developed as a therapeutic regimen for pancreatic dysfunction.

The data to be presented demonstrate the feasibility of direct imaging of ß-cell (ßTC3) activation in the presence of Mn^{2+}. ICP mass spectroscopy and MRI results confirm that Mn^{2+} acts as a calcium analog in ß-cells and can therefore be used as a contrast agent. The Mn^{2+} doses examined were based on preliminary studies of saturation kinetics of isolated cells, and utilized subtoxic levels in obtaining MRI images. We also have successfully demonstrated high-resolution functional imaging of intact islets. Preliminary data suggest that islets are more efficient in Mn^{2+} uptake than insulinoma cells and can therefore be exposed to much lower doses of Mn^{2+} to obtain T_1-weighted images. Long-term, periodic evaluation of beta cell function by this technique can be used to optimize implant parameters and conditions. This provides the promise of MR tagging of islets prior to transplantation. However, to tag the islets with Mn^{2+}, a better understanding of Mn^{2+} efflux from islets is required.

It is hoped that the proposed combination of the non-invasive high-resolution characteristics of MRI and the ability to directly measure ß-cell activation will provide a powerful tool for understanding diabetes and for evaluating emerging therapeutic technologies.

Ho, Chien

Molecular and Physiological Imaging Using MRI
Chien Ho
Carnegie Mellon University

In this presentation, we shall give a brief summary of our recent research using a two-pronged approach to investigate graft rejection following organ transplantation in our rodent transplantation models. First, by labeling immune cells, such as T-cells and macrophages, with a magnetic resonance imaging (MRI) contrast agent, dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) particles, we can use MRI to monitor the accumulation of these labeled immune cells at the rejecting graft as a non-invasive method to detect graft rejection. The cells can be labeled ex vivo and then introduced into the animal or MRI contrast agents can be introduced directly into the animal. Second, we can monitor the function of a transplanted organ by MRI during various stages of the rejection process. For rat kidney transplantation, we can use MRI to evaluate first-pass renal perfusion with USPIO particles. Our results show good agreement between the renal graft perfusion measurements and histopathological changes associated with rejection. Using a novel heterotopic working rat heart model, we plan to establish functional indexes from cine MRI data that correlate with cardiac rejection. Our present results show that diastolic functions deteriorate earlier than systolic functions during rejection. In addition, we have found that regional dysfunction in early rejection can be detected by MRI tagging.

The experimental findings have demonstrated that our MRI-based approach is capable of detecting acute rejection in a quantitative manner. This two-pronged approach monitors not only immune cell infiltration, but also organ dysfunction accompanying graft rejection. Thus, multi-parameter measurements avoid sampling errors and can make accurate and non-invasive diagnosis of graft rejection possible.

Achilefu, Samuel

Design of Optical Molecular Imaging Agents

Samuel Achilefu and Sharon Bloch
Washington University School of Medicine

Molecular probes play a major role in our understanding of the molecular basis of pathogenesis. With the recent advances in molecular imaging methods, these probes, some of which were initially developed for ex vivo assays, have been adapted to monitor or image molecular events in vivo. Due to its high sensitivity, nuclear methods have been the dominant molecular imaging modality in humans and hence, a variety of radiopharmaceuticals has been developed. While the sensitivity of paramagnetic contrast agent-mediated magnetic resonance imaging is low, recent studies have demonstrated that physiological processes can be imaged at the molecular level with “smart” MRI contrast agents or nanoparticles. A complementary and viable alternative to conventional imaging systems is the emerging optical imaging method that has the capability of detecting minute amount of tracers, as with nuclear methods. Although a plethora of molecular probes has been developed for in vitro assays by spectroscopy or microscopy, adaptation of these probes to in vivo applications remains an ongoing challenge. This talk will focus on the design of optical and other imaging agents and will address their potential application in the imaging of pancreatic beta cells.

Clark, Paige

PET Imaging of the Normal Pancreas with [18-F]-4-Fluorobenzyltrozamicol(FBT), a Cholinergic Tracer

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Wake Forest University Health Sciences

The pancreas is one of the most heavily innervated organs in the neuroendocrine system. For example, cholinergic neurons that terminate in the pancreas provide tight control over beta cell functions. [18-F]-4-fluorobenzyltrozamicol (FBT) is a PET radiotracer that binds to the vesamicol receptors on the acetylcholine vesicles of the presynaptic component of both muscarinic and nicotinic ganglia. FBT has been best studied in the brain and spinal cord, where FBT binding has correlated with acetylcholine (Ach) vesicular density. The use of neuro-receptor radiopharmaceuticals for pancreatic imaging by SPECT or PET is a neglected area of research.

The purpose of this study was to determine whether the pancreas can be imaged by FBT PET. Weight appropriate doses of [18F]-FBT were injected intravenously in 3 adult rhesus monkeys (2 male and one female), one adult male human, and 6 adult male Balb/C mice. For the primates, serial abdominal scans were performed for 90 minutes using a clinical PET scanner (GE or Siemens). Mice were imaged one hour post injection on a Concorde P-4 microPET scanner. Primate images were acquired and processed using attenuation correction facilitated by transmission scans. For 2 of the rhesus monkeys, spiral CT was also performed over the same distribution as PET and displayed in 2.5mm slices, with coronal and sagittal 2D reconstruction. PET and CT images were compared visually.

In the mammalian species examined, the pancreas was intensely FBT-avid, with uptake greater than was observed by any other organ, including the brain. Excellent pancreatic uptake of FBT occurs over a permissive, prolonged interval, from 10-90 minutes post injection. These data suggest that neuro-receptor imaging with FBT PET, or more sub-selective neuro-receptor ligands, may be useful for non-invasive quantification of specific functional pancreatic elements.

Constantinidis, Ioannis

Use of NMR Techniques to Study Insulin Secreting Cells

Ioannis Constantinidis, Nicholas E. Simpson, Cheryl L. Stabler, Samual C. Grant, Robert C. Long,Jr., Steven J. Blackband, and Athanassios Sambanis
University of Florida

The bioartificial pancreas is potentially an efficacious treatment for diabetes that can provide physiologic blood-glucose regulation without immunosuppressive medication. Although various designs have been considered, the design most commonly used to generate these constructs is based on the microencapsulation of insulin-secreting cells in a biocompatible matrix that provides mechanical support and, at least, partial immunoprotection. A variety of cells have been used in these constructs including mammalian islets and transformed beta-cell lines, and the matrix most frequently utilized is the alginate/poly-L-lysine/alginate bead. At present, our only means of assessing the efficacy for an implanted bioartificial pancreas is to measure the blood glucose concentration of the host. Developing a non-invasive imaging technique that can monitor the viability and function of encapsulated cells as well as the integrity of the matrix is of critical importance. Nuclear Magnetic Resonance (NMR) has the ability to provide both biochemical and structural information, under either in vivo or in vitro conditions.

^{1}H NMR imaging and spectroscopy is being used to obtain data from alginate encapsulated beta cells under both in vitro and in vivo conditions. In vitro, T_2 and diffusion-weighted NMR imaging (32x32x200 micron pixel resolution) was used to locate the position and distribution of cells within the capsule, while ^{1}H NMR spectroscopy localized within a single bead (voxel volume less than 300 nanoliters) was used to monitor intracellular metabolites. In vivo, the same imaging pulse sequences, albeit at lower spatial resolution, were used to identify the accumulation of peritoneal cells around an implanted construct while localized ^{1}H NMR spectroscopy was used to monitor "Choline" signal intensity from encapsulated beta cells implanted in the peritoneal cavity of B6 mice.

In addition to these studies, ^{13}C NMR spectroscopy is being applied on cellular extracts to study the correlation between glucose metabolism and insulin secretion. As a first step in these experiments we investigated the role of media composition in glucose utilization and insulin secretion. For these experiments uniformly labeled glucose and an isotopomer analysis of the glutamate resonances were used on three different insulin secreting beta cell lines. Our data show that changes in medium composition alter the isotopomer patterns of the glutamate resonances and thus the relative fluxes through the key entries to the TCA cycle. Furthermore, these changes in medium composition induce changes in the rates of glucose consumption and insulin secretion independent of glucose concentration. Understanding the role of medium composition during the culture of the cells is critical in reaching the correct, physiological, conclusions regarding the mechanism of insulin secretion.

Overall, NMR (both imaging and spectroscopy) is an excellent modality to monitor biochemical and structural information for either free or encapsulated beta cells, under in vivo and in vitro conditions.

Evaluation of [C-11]Glyburide as a Pancreatic β-Cell Imaging Agent

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Sulphonylurea is widely used to treat type II diabetes. It stimulates insulin release by binding to the pancreatic beta cell membrane and blocking the ATP-sensitive potassium ion efflux. It binds to the ß-cell membrane with high affinity. These binding sites have been named sulphonylurea receptors (SUR). We are interested in the development of a positron-labeled ligand for monitoring the SUR in vivo, which will provide a novel approach for probing the functions of pancreatic cells.

The lead compound for tracer development is glyburide, which has a K_D of 0.3 nM for the SUR. In vitro studies have shown that the uptake of [H-3]glyburide is proportional to the number of beta cells and the uptake is saturable. These characteristics strongly suggested that positron-labeled glyburide might be a potential tracer for imaging beta cells in vivo. Previously, radiolabeled glyburide analogs, such as [I-123]iodoglyburide and [F-18]fluoroethoxyglyburide, have been evaluated. To our knowledge, there are no reports on the in vivo biodistribution of these tracers. Our aim is to evaluate the potential of [C-11]glyburide as a PET tracer for imaging pancreatic beta cells.

Glyburide was synthesized according to literature procedure and its O-demethylated precursor was prepared by O-demethylation of glyburide. All compounds were characterized by HNMR and confirmed by high-resolution mass spectrum. [C-11]Glyburide was prepared by reacting the precursor with [C-11]methyltriflate in DMF. The crude product was purified by semi-preparative HPLC. The synthesis time was about 30 min and the radiochemical yield was 8% at end-of-synthesis (EOS). The specific activity was 1.5 mCi/nmol at EOS.

Initial biodistribution studies were performed using male Sprague Dawley rats, which were divided into 4 groups (n = 3 per group): 5 min, 15 min, 45 min and 45 min co-injection (1 mg glyburide/kg). Each animal received 0.1 mCi of the tracer via tail vein injection. At the specified time point, the animal was sacrificed by decapitation and organs were removed, weighed, and counted. The uptake was expressed as percent injected dose per gram of tissue (%ID/g). Initial results indicated that liver has the highest uptake (8%), which is followed by kidney (1%). Low uptake was observed in the pancreas (0.1%). Tissue uptake was not affected by the co-injection of glyburide, suggesting tissue uptakes were mainly nonspecific. Our initial results suggest that [C-11]glyburide is not a good tracer for imaging beta cells.

Imaging Molecular Expression with In Vivo confocal and Two Photon Microscopy

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Intravital microscopy is a powerful method for visualizing cellular interactions in the native environment of living animals. However, conventional (epifluorescence) intravital microscopy has limited ability to resolve the three-dimensional tissue architecture. We have developed a confocal/two-photon fluorescence microscope that is capable of taking high resolution images (optical sections) in vivo, up to several hundred microns deep into tissue. Using fluorescent antibodies against specific cell surface markers, we are able to image molecular expression on vascular endothelial cells noninasively through the intact skin of live mice. Internal organs such as the pancreas can be imaged by exposing the tissue surgically. In addition to fluorescent antibodies, specific enzymes activable probes and green fluorescent protein reporter probes allow functional imaging of the cellular microenvironment. Two photon microscopy is particularly useful for imaging the short wavelength green fluorescent proteins with near-infrared excitation, while long wavelength probes such as Cy5.5 can be linked to antibodies and other targeting molecules for imaging with confocal microscopy. Rapid scanning allows realtime (video rate) imaging of circulating cells in the vasculature.

Lu, Yuxin

Repetitive microPET Imaging of Implanted Human Islets in Mice

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There is currently no method to visualize islet grafts non-invasively. If a non-invasive imaging technology were to become available, clinicians could monitor the survival of transplanted islets, evaluate the efficacy of therapies designed to prolong allograft survival, and make changes in the protective regimen as needed. The UCLA Gene Imaging Consortium has developed a microPET scanner for the imaging of small animals with a spatial resolution approaching ~1^3 mm^3. They have also pioneered the development of PET reporter genes. With this technology, tissues that express a PET reporter gene can be quantitatively imaged repeatedly in the same animal in a non-invasive fashion.

As a first step towards imaging islet grafts, we performed a feasibility study using NIT cells. Ten million NIT cells were transduced with adenovirus (Adeno-TKm) engineered to express a mutant Herpes Simplex Virus Type 1 thymidine kinase driven by CMV and then implanted under the shoulder blade of a NOD-scid mouse. Using [18F]-FHBG (a penciclovir analog) as a imaging probe, a significant PET signal was readily detected from the implant. Next, we infected 5000 human islet equivalents with Adeno-TKm and implanted them into the axillary cavity of a NOD-scid mouse. A moderately strong signal (0.82% of injected dose) from the graft was detected with microPET. This animal was repeatedly imaged with microPET over a 30-day period, during which the PET signal decreased, presumably due to the transient nature of adenovirus expression. Immunohistological analysis of the islet graft showed that adenovirus infection and thymidine kinase expression had no apparent affect on islet insulin, glucagon or somatostatin content.

These data demonstrate that human islets can be engineered to express PET reporters and can be monitored non-invasively by PET after implantation. However, adenovirus directs only the transient expression of the reporter genes as expected. We are now extending these initial studies using recombinant lentivirus and AAV vectors that should allow prolonged expression of PET reporters and long-term islet imaging.

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Malaisse, Willy J.

Possible use of D-Mannoheptulose for the Non-invasive Imaging of Insulin-producing Pancreatic Islet Cells

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It was recently proposed that the seven-carbon ketoheptose D-mannoheptulose could represent a suitable tool for the non-invasive imaging and quantification of insulin-producing pancreatic islet cells. This proposal was based on the finding that D-mannoheptulose is transported into cells at the intervention of GLUT2 [1]. In support of such a proposal, it was documented that the measurement of tritiated D-mannoheptulose uptake allows to quantify the total mass of islet β -cells in either the isolated perfused rat pancreas (comparison between control rats and streptozotocin-induced diabetic animals) or isolated islets from control rats, streptozotocin-induced diabetic animals and hereditarily diabetic GK rats. The potential objection that, in human pancreatic islets, GLUT1, rather than GLUT2 represents the major carrier system for the transport of monosaccharides across the B-cell plasma membrane was answered by investigations on the effects of D-mannoheptulose upon D-glucose metabolism and insulinotropic action and on the uptake of tritiated D-mannoheptulose in human islets. Further work in this perspective aimed at selecting the most appropriate probe between the following candidates : ^{11}C- or ^{13}C-labelled D-mannoheptulose, 3-deoxy-3-[^{18}F]fluoro-D-mannoheptulose, 1-deoxy-1-[^{123}I]iodo-D-mannoheptulose or 7-deoxy-7-[^{123}I]iodo-D-mannoheptulose. The two last candidates offer the advantages of using a radioactive isotope with a longer half-life and suitable for imaging by single photon emission computerized tomography. A longer half-life may favour the ratio between intracellular and extracellular radioactive content, since D-mannoheptulose is phosphorylated by both low-K_m hexokinase and glucokinase and that the radioactive acidic metabolites of tritiated D-mannoheptulose remains inside dispersed islet cells even after extensive and prolonged washing. Experiments conducted with 1-deoxy-1-[^{125}I]iodo-D-mannoheptulose suggested, however, that it did no more display the same specificity towards GLUT2 as tritiated D-mannoheptulose. By analogy with the use of ^{125}I- or ^{123}I-labelled 6-deoxy-6-iodo-D-glucose as a tracer for the in vivo or in vitro determination of D-glucose transport in different cell types, 7-deoxy-7-[^{123}I]iodo-D-mannoheptulose could, therefore, represent the best candidate for the non-invasive imaging of insulin-producing cells. The present steps in this perspective now consist in the synthesis of the latter iodinated heptose and the identification of the phosphorylation site in the D-mannoheptulose molecule.

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Markowitz, Zvi

Human vs. Machine Estimates of Beta-Cell: What are the Tradeoffs?

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When experts estimate beta-cell area or volume, we assume that they identify (label) the cell type perfectly at every point in the image. The number of points they can count, however, is limited. By contrast, if automated image analysis is used, all the points will be counted but labeled imperfectly due to imperfections of the image-processing algorithm.

This leads to the following question: For a given level of measurement error, how can we decide whether to use a human or machine approach? Clearly, it depends, on one hand, on the error rate of the automated classifier, and on the other hand on the number of points the human is willing to count.

The basis of stereology is the combination of two processes: labeling and counting. This work examines the theoretical and practical considerations in choosing between manual and image processing-based stereology; in particular, we derive and present a measure that combines the classification (labeling) and sampling (counting) errors. This measure allows the user to choose a method that optimizes overall performance (as expressed by the variance of the quantity to be measured), given the human and machine capabilities available.

Given, for example, the number of slices and the number of points per slice used in estimating volume, the user finds those values on a set of curves, and reads out a criterion value. That value is compared to the performance of a proposed automated classifier; the outcome of the comparison determines which method will yield a smaller measurement variance.

The method is illustrated and validated with synthetic cases (the Cavalieri method applied to a sphere and a cube) and with real beta-cell photomicrograph data.

McClanahan, Timothy

Automated Segmentation of Rat Pancreas in Color Photomicrographs

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In this paper, we present an image processing technique that reliably segments rat pancreatic tissues from in vitro color photographic images of pancreas thin sections. The algorithm will automatically classify insulin stained images for fat, islets, open space, and pancreas tissue. The system utilizes morphological, edge detection and gradient descent, image processing techniques to identify feature threshold positions in each image that initially segments tissue classes. A neural network identifies segmented islet cells by textural and color differences between internal and locally external beta cell regions. The system is written in the Interactive Data Language 5.4 (IDL) and was evaluated against 200+ insulin stained rat pancreas images. The images are to be evaluated against several other automated segmentation techniques. This information can be used in conjunction with stereologic procedures to determine pancreas beta-cell mass (BCM), and tissue distributions which are used to infer important information about cell morphology, and insulin quantity.

Paty, Breay

Beta cell Specific ^{125}I-labeled IC2 Antibody is a Promising Method of Measuring Intrahepatically-transplanted Islet Mass In Vivo

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Restoration of endogenous insulin secretion in type 1 diabetes using islet transplantation has shown great promise recently. To assess the efficacy of this emerging treatment, an accurate in vivo measure of beta cell mass is needed. Recently, a method of measuring in vivo beta cell mass in the native pancreas using a ^{111}I-labeled beta cell-specific antibody was demonstrated to be useful in streptozotocin-treated mice. We hypothesize that this method can also be used to assess beta cell mass post-islet transplantation. Consequently, we purified an IgM antibody directed against the beta cell-specific IC2 surface antigen, then labeled the antibody with ^{125}I and incubated it for 1-hour with freshly isolated, handpicked islets obtained from Balb-c mice using standard collagenase digestion. After incubation, the labeled islets were washed and transplanted intrahepatically into recipient Balb-c mice using intraportal injection technique. Recipient mice were transplanted either with 200 islets (n=3), or 500 islets (n=3) each. Two hours after transplantation, the mice were sacrificed and all organs were resected and radioactivity counted using a scintillation counter. The mean total body counts in animals that received 200 vs. 500 islets were 3987 1032 dpm vs. 8707 1160 dpm, respectively (ratio: 2.2, p = 0.038). The liver counts in animals that received 200 islets vs. 500 islets were 1904 419 dpm vs. 4714 680 dpm respectively (ratio: 2.5, p = 0.024). The ratio of counts in the liver (1 : 2.5) almost exactly reflects the total ratio of transplanted islets in each group (200 : 500). These early results show that intrahepatically transplanted islet mass can be quantitatively assessed using this technique. We conclude that it is possible to assess transplanted islet mass using labeled beta cell specific antibodies and that further studies examining the utility of intravenous injection of labeled antibody has great potential as a way of assessing transplanted islet mass in vivo.

Sambanis, Athanassios

Development and Non-invasive Monitoring of a Pancreatic Tissue Substitute

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A pancreatic tissue substitute for treatment of insulin-dependent diabetes has significant potential in providing a less invasive, more physiologic regulation of blood glucose levels than daily insulin injections. We are pursuing the development of core, enabling technologies for pancreatic substitutes based on continuous beta pancreatic lines and on potentially autologous non-beta cells. The enabling technologies depend strongly on the type of cells used. With allogeneic cells, development of encapsulated systems for immune protection and in vivo immune acceptance and function are of critical importance. With non-beta cells, genetic engineering for secretion of recombinant insulin in response to physiologic stimuli is essential for success of this approach. In both cases, monitoring of constructs in vivo is significant in order to provide a link between implantation and end-point physiologic effects. We are investigating the use of NMR imaging and spectroscopy as non-invasive modalities for assessing construct function post-implantation.

Systems of encapsulated mouse insulinomas are designed using diffusion/reaction models with experimentally measured parameter values and are characterized in vitro for secretion dynamics with a microperfusion apparatus and for long-term function with an NMR-compatible perfusion bioreactor. Results suggest that the encapsulated insulinomas constitute a dynamic construct, which stabilizes at a metabolic and secretory plateau determined by the available oxygen in the surrounding milieu. The in vitro NMR studies are being expanded to monitoring constructs non-invasively in vivo. For this, an agarose-based, macro-encapsulated construct design is implemented, so that ^{1}H NMR imaging and localized ^{1}H NMR spectroscopy on implanted cells can be performed. Initial results indicate that choline levels, measured by ^{1}H NMR spectroscopy, may indeed track the number of viable cells in constructs. These studies also help define the parameters in three-dimensional construct architecture, which are necessary for successful in vivo NMR monitoring.

Work with non-beta cells focuses on cells of hepatic and enteroendocrine origins. Hepatocytes can be genetically engineered to produce insulin under transcriptional glucose regulation, and their secretion dynamics can be significantly improved by destabilizing preproinsulin mRNA. Still, these cells may not exhibit the acute insulin secretion dynamics needed after consumption of a meal for euglycemia in higher animals and, eventually, humans. Recent work with enteroendocrine L cells demonstrated that these cells can indeed acutely secrete recombinant insulin along with endogenous glucagon-like peptide-1 in response to stimulation from meat hydrolysate. The possibility of using NMR imaging and spectroscopy to assess the function of tissue constructs based on such potentially autologous cells will be discussed.

Shiue, Chyng-Yann

Synthesis of F-18 Labeled Glyburide Analogs as Beta-cell Imaging Agents

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Diabetes mellitus is characterized by a deficiency in insulin secretion and beta-cell loss. A non-invasive method to monitor the status of beta-cells during the silent phase of prediabetes or after pancreas transplantation will be useful for the treatment of diabetes mellitus. The purpose of this study was to develop fluorinated glyburide analogs [2-fluoroethoxy (3), 2-fluoropropoxy (4), 2-fluorobutoxy (5), 2-p-fluorobenzyl-5-chloro-glyburide (6) and their 5-iodo analogs (7-10)], and study their structure-activity relationships as insulin secretagogues and beta-cell imaging agents.

Compounds 3-10 were synthesized in multi-steps in ~ 10% yield. The F-18 labeled 3-5 and 7-9 were synthesized by alkylation of hydroxyglyburide with the appropriate F-18 labeled fluoroalkyl tosylate followed by purification with HPLC in 5-10% yield in a synthesis time of 120 min from EOB. These compounds were evaluated as insulin secretagogues and beta-cell imaging agents. The results showed that increasing the length of the side chain of the fluorinated glyburide analogs resulted in an increase of their non-specific binding to whole beta-cells and that F-18 labeled compounds 3, 7 and 6, 10 may serve as beta-cell imaging agents.

Sweet, Ian R.

Screening for Beta Cell Imaging Agents

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The beta-cell loss seen in Type I and II diabetes could be monitored clinically by positron emission tomography (PET) if imaging agents were sufficiently specific for islet cells to overcome the high ratio (=100:1) of exocrine-to-islet tissue in pancreas. The daunting criteria highlights the need for a systematic approach to quantitatively screening compounds based on specificity. As a screen for beta-cell specific compounds, we compared accumulation and retention by islet, INS-1 and exocrine (PANC-1) cells of candidate molecules. Two cell types were chosen as the model for beta cell: INS-1 cells and isolated rat islets. INS-1 cells allow for evaluation of compounds against a homogeneous cell population, although it is recognized that there are quantitative differences between INS-1 cells and native beta cells. Rat islets have the disadvantage of containing cell types other than beta cells, and diffusional space in the islet that prevents the rapid access of imaging agents to beta cells. Molecules tested include glibenclamide, tolbutamide, serotonin, L-DOPA, dopamine, nicotinamide, fluorodeoxyglucose, and dithizone. Accumulation of radioactivity in the screen was determined by incubating cells with radiolabeled molecules in KRB at 37°C for 30 min, spinning cells through an oil layer, and counting the radioactivity in the cell pellet. Retention of radioactivity was determined by washing cells following the 30-minute labeling period, further incubating cells in label-free media for 40 min and then counting the radioactivity in the cell pellet. These times points were chosen for the screen based upon kinetic studies of uptake of these compounds which revealed that they all had half times less than 30 minutes. Therefore, a screen could be carried out with a single time point that would adequately represent the uptake and retention by the cell. Accumulation in INS-1 cells and islets were highest for glibenclamide, nicotinamide, serotonin, dopamine and dithizone (ratio of uptake relative to water > 5). Only nicotonamide was retained by islet cells with a ratio relative to water greater than 5. Data was represented as a ratio of uptake or retention by beta cells relative to PANC-1 cells, and only glibenclamide had a ratio that was greater than 10. Although, the extrapolation of in vitro data to in vivo conditions has not been validated in this study, with a group of candidates that are known to have a high affinity for beta cells, the specificity ratios were not close to the goal of more than 100 for any compound. An additional hurdle is the 30 to 1 extracellular water:beta cell water space, which is an especially stringent criteria for compounds like sulfonylureas that bind to proteins in the blood. The simplicity of this cell-based screening method offers advantages to whole-animal and whole-organ studies that are harder to perform and interpret. Establishment of standard methods with which to evaluate candidate molecules will allow comparison between laboratories and pooling data in order to establish a relation between in vitro screening and specificity in in vivo studies.
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Virostko, Jack M.

Quantification of Transplanted Beta Cells Using In Vivo Bioluminescent Imaging

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Non-invasive and quantitative assessment of presence and function of transplanted beta cells continues to pose a formidable challenge. In Vivo Bioluminescence (IBL) has been touted as a novel method of imaging in vivo molecular and cellular function. While many studies are starting to use this method, quantification and subsequent correlation of detected light emission to biological processes has thus far been treated in an overly simplified way. The aim of this study is to characterize the validity and sensitivity of IBL for the specific application of imaging transplanted human pancreatic islets. Preliminary results indicate that IBL of human beta cells transfected with the firefly reporter gene (luciferase) and subsequently transplanted under the kidney capsule of NOD-SCID mice, is feasible and can be quantified using an ultra sensitive, liquid nitrogen-cooled , back-illuminated, back-thinned, CCD camera (Roper Scientific, EEV 1300). Bioluminescent islets in three doses (500, 1000, and 2000 islets) were transplanted and imaged weekly for 4 weeks post transplantation. Weeks following transplantation the islet grafts showed a stable, linear relationship between number of islets and intensity of bioluminescence. This study indicates that bioluminescence can be used to quantify the number of islets remaining post transplantation, as well as track the time course of islet engraftment. In order to validate our imaging technique and quantify the light absorption by tissues overlying the renal capsule, we implanted mice with light emitting rods. These rods consist of glass capillaries (0.9 mm diameter and 2 mm long) filled with tritium (a beta emitter with a half life of over 10 years) which excites a phosphor that emits light at 600 nm. These implanted beads simulate a constant source of bioluminescence from a known location. Positioning of the mouse for imaging was optimized using these beads. Lateral placement provided the shortest path of light propagation through the mouse and subsequently the highest intensity signal. Shaving the mouse above the renal capsule was also found to increase detected light intensity. Shaving removed the scattering and absorption caused by hair and increased detected luminescence by 50%. This combination of lateral placement and shaving also lowered the variability inherent in placing the mouse in the imaging chamber. Slight alterations in the position of the mouse cause some variation in the detected luminescence; however, with shaving and lateral placement this variability was reduced to 5%. The bead implanted mice were imaged at various time points post transplantation in order to investigate the effects of fibrosis and surgical scar tissue above the renal capsule on the detected bioluminescence intensity. Both of these factors lower the detected bioluminescence intensity in mice with transplanted beta cells by absorbing light. Scar tissue was found to attenuate luminescence greatest immediately post surgery, then gradually less as the skin above the renal capsule heals. This finding indicates that quantification of IBL must take into account the post-surgical effects on tissue overlying the bioluminescent source.

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