Review

MOLECULAR METABOLISM

Intraocular *in vivo* imaging of pancreatic islet cell physiology/pathology

Ingo B. Leibiger**, **Per-Olof Berggren***

ABSTRACT

Background: Diabetes mellitus has reached epidemic proportions and requires new strategies for treatment. Unfortunately, the efficacy of treatment regimens on maintaining/re-gaining functional beta cell mass can, at the present, only be determined indirectly. Direct monitoring of beta cell mass is complicated by the anatomy of the endocrine pancreas, which consists of thousands to a million of discrete micro-organs, i.e. islets of Langerhans, which are scattered throughout the pancreas.

Scope of review: Here, we review the progress made over the last years using the anterior chamber of the eye as a transplantation site for functional imaging of pancreatic islet cells in the living organism. Islets engrafted on the iris are vascularized and innervated and the cornea, serving as a natural body-window, allows for microscopic, non-invasive, longitudinal evaluation of islet/beta cell function and survival with single-cell resolution in health and disease.

Major conclusions: Data provided by us and others demonstrate the high versatility of this imaging platform. The use of 'reporter islets' engrafted in the eye, reporting on the status of *in situ* endogenous islets in the pancreas of the same animal, allows the identification of key-events in the development and progression of diabetes. This will not only serve as a versatile research tool but will also lay the foundation for a personalized medicine approach and will serve as a screening platform for new drugs and/or treatment protocols. 'Metabolic' islet transplantation, in which islets engrafted in the eye replace the endogenous beta cells, will allow for the establishment of islet-specific transgenic models and 'humanized' mouse models as well as serving as the basis for a new clinical transplantation site for the cure of diabetes.

Keywords Diabetes mellitus; Type 1 diabetes; Type 2 diabetes; Pancreatic islet; Pancreatic beta cell; Live-cell imaging; Fluorescence microscopy; *In vivo* imaging; Anterior chamber of the eye

1. INTRODUCTION

Diabetes mellitus has reached epidemic proportions with increasing numbers of patients with both Type 1 (T1DM) and Type 2 diabetes (T2DM). In addition to the 415 million adults who are estimated to currently have diabetes, there are 318 million adults with impaired glucose tolerance, which puts them at high risk of developing the disease in the future and will contribute to the 642 million diabetes patients expected by 2040 [1]. Moreover, the International Diabetes Federation estimates that currently 193 million people suffering from the disease are undiagnosed and, consequently, at risk of developing diabetes complications.

T1DM is caused by an autoimmune destruction of insulin-producing pancreatic beta cells. However, the presence of circulating C-peptide in T1DM patients with a long history of the disease [2] suggests that some beta cells either escape the immune attack or that new beta cells are generated.

The majority of diabetic patients suffer from T2DM, a condition that develops when pancreatic beta cells fail to provide the organism with sufficient amounts of insulin to keep blood glucose levels within physiological limits. The relative insulin deficiency in T2DM is caused by beta cell failure to produce/secrete sufficient amounts of insulin to overcome peripheral insulin resistance mainly in skeletal muscle, fat, and liver.

Consequently, for both T1DM and T2DM, treatment strategies have to be developed that aim at protecting, preserving, or re-establishing functional beta cell mass. Unfortunately, the efficacy of treatment regimens on maintaining/re-gaining functional beta cell mass at present can only be determined indirectly, i.e. by measuring blood glucose levels in combination with circulating insulin/C-peptide levels. Direct monitoring of beta cell mass is complicated by the anatomy of the endocrine pancreas, which consists in humans of approximately 1 million discrete micro-organs, i.e. islets of Langerhans, scattered throughout the pancreas. In addition, beta cell mass can be highly variable between healthy individuals, making it impracticable to use an absolute number as a diagnostic tool. On the other hand, non-invasive, long-term monitoring of functional beta cell mass within the same individual will be an important step in the direction of a personalized medicine approach to combat this disease. A recent review describes in detail the current state in the generation of imaging probes and

The Rolf Luft Research Center for Diabetes and Endocrinology, L1:03 Karolinska Institutet, SE-171 76 Stockholm, Sweden

*Corresponding author. E-mail: per-olof.berggren@ki.se (P.-O. Berggren).

**Corresponding author. E-mail: ingo.leibiger@ki.se (I.B. Leibiger).

Received January 31, 2017 • Revision received March 7, 2017 • Accepted March 18, 2017 • Available online 4 May 2017

http://dx.doi.org/10.1016/j.molmet.2017.03.014

respective imaging modalities, e.g. magnetic resonance imaging (MRI), positron emission tomography/single-photon emission computed tomography (PET/SPECT), and bioluminescence imaging [3]. While these techniques will eventually allow quantification of beta cell mass *in vivo*, they will not enable quantification of subtle changes at the sub-islet level due to lack of spatial resolution. Such changes include changes in intra-islet blood flow, innervation, and beta cell heterogeneity in function and survival. As optical imaging techniques including confocal microscopy and optical coherence tomography to monitor pancreatic islet function have been discussed [4,5], here, imaging was often combined with either the exteriorized pancreas approach or with implantation of an optical body-window. The latter allows monitoring pancreatic islet function only for a few weeks.

In 2008, we presented an imaging approach that allows for monitoring pancreatic islet function and survival non-invasively, longitudinally at single-cell resolution in the living animal [6,7]. We transplanted isolated islets into the anterior chamber of the eye (ACE), where they engraft on the iris and become innervated and vascularized. By using the cornea as a natural body-window, these islets are readily available for functional microscopic imaging.

The ACE has been a transplantation site for a long time. van Dooremaal first described introducing/implanting different types of objects (e.g. pieces of paper) and tissues (e.g. pieces of skin) into the ACE of dogs and rabbits in 1873 [8]. Since then, the ACE has been used as a transplantation site for a variety of tissues ranging from ovaries [9], prostatic tissue [10], peripheral and central nervous tissues [11–13], kidney glomeruli [14], and pancreatic tissue [15–18]. With this information at hand, we hypothesized that the ACE should represent a unique transplantation site for pancreatic islets utilizing the cornea as a natural body-window for functional *in vivo* imaging of islet cells.

In the present review, we discuss the progress that has been made over the last years using this approach to study pancreatic islet/beta cell function and survival in health and disease. A schematic illustration summarizing the use of the ACE-based *in vivo* imaging technique is given in Figure 1.

2. PANCREATIC ISLET TRANSPLANTATION TO THE ACE

2.1. The procedure

The procedure of islet transplantation into the ACE of mice is described in great detail in a step-by-step protocol [7] as well as in a video tutorial [19]. In brief, pancreatic islets are isolated by standard procedures by either collagenase digestion or micro-dissection and aspirated into a blunt 27-G cannula in a minimal volume (10–20 μl). The anesthetized recipient-mouse is placed under a stereomicroscope, and the cornea is punctuated with the cannula between apex and limbus of the cornea. After gently inserting the cannula into the ACE, the islets are placed onto the iris by slow injection. The above-mentioned papers give a detailed layout of the experimental design including choice of donor and recipient mice, choice of anesthetics, as well as a description of used materials and equipment. Full engraftment of islets takes place within four weeks after transplantation. Engrafted islets can be monitored by various microscopic techniques ranging from simple light microscopy, fluorescence microscopy to high-resolution laser-scanning confocal microscopy/two-photon laser scanning microscopy.

In general, a syngeneic transplantation strategy is preferred; however, the broad range of potential donor mice with desired genetic modifications and a different genetic background requires an allogeneic approach and thus the use of immune-deficient recipient mice (e.g. nude mice, Rag1$^{-/-}$ mice, or NSG mice) to avoid immunological responses. Similarly, using immune-deficient mice as recipients is required when performing xenotransplantation to generate a 'humanized' mouse model (see section 2.4.2.). Given that the eye is an immune-privileged site, the need for immune-deficient mice might seem counterintuitive. However, it should be stressed that during the process of islet vascularization in the ACE, immune-privilege is broken. This observation allows for studying immune processes involved in islet graft rejection or mechanisms involved in T1DM development (see section 3.). The fact that the ACE can also be used for immunological studies is of particular importance when trying to identify novel and efficient strategies for intervention.

2.2. Islet vascularization in the ACE

In the pancreas, pancreatic islets are interspersed by a dense network of capillaries that guarantees the efficient exchange of oxygen, nutrients, and hormones between endocrine cells and the blood circulation of the body. Vascularization of the islets engrafted in the ACE occurs from the iris, which has a rich vessel-bed. When studying the dynamics of islet re-vascularization [6], at day 3 after transplantation, we observed that in the vicinity of sites where islets were attached to the iris, structural rearrangements of iris vessels took place. Seven days after transplantation, blood vessels continued to grow and by day 14 formed a micro-vascular network throughout the graft. From two to four weeks after transplantation, the vascular network became denser. By the end of four weeks, the network reached a plateau and was characterized by uniformly sized capillaries. The diameter of blood vessels started to decrease three days after transplantation and, by the end of four weeks, reached a diameter that was similar to that of the intra-islet vasculature in the pancreas. When transplanting islets freshly after isolation, these islets contain a substantial amount of intra-islet endothelial cells. These donor-islet endothelial cells contribute to the early events of the re-vascularization process by increasing the re-vascularization rate but do not increase the vascular density of the graft at four weeks after transplantation when compared to islets that were kept in culture and which had lost the majority of their endothelial cells [20]. Hence, both donor and host endothelial cells are capable of forming functional capillaries in the engrafted islets. As within the pancreas, islets engrafted in the ACE show a normal ultrastructure, i.e. endocrine cells and endothelial cells are separated by a single basement membrane and capillaries formed by thin endothelial cell bodies with fenestrations covered by a thin diaphragm [20]. The dynamics and the quality of the re-vascularization process are age-dependent [21]. Almaca et al. [21] demonstrated that islets of 18 months old mice showed the same blood-vessel density as islets from two months old mice; however, the aged islets had inflamed and fibrotic blood vessels. Moreover, the aged islets contained twice the number of macrophages, which were associated with blood vessels showing increased expression of intercellular adhesion molecule 1 (ICAM-1). In addition, these islets had higher expression levels of macrophage colony-stimulating factor receptor (CSFR1) and vascular cell adhesion molecule 1 (VCAM1). When transplanted into the ACE of two months old recipient mice, the re-vascularization of aged islets was delayed by one month when compared to young islets. Aged islets had lower vessel densities than younger islets in the first month after transplantation but showed noticeable re-vascularization within the following months. In addition to the delayed initial vascularization process, aged islets contained larger vessels that did not branch-out as much as vessels in young islet grafts. Between months three and seven after transplantation, aged islets showed regions of newly formed capillaries with smaller diameter, which allowed rejuvenation and functional recovery. It is noteworthy that transplantation of the

