

Imaging of β -Cell Mass and Insulinitis in Insulin-Dependent (Type 1) Diabetes Mellitus

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Insulin-dependent (type 1) diabetes mellitus is a metabolic disease with a complex multifactorial etiology and a poorly understood pathogenesis. Genetic and environmental factors cause an autoimmune reaction against pancreatic β -cells, called insulinitis, confirmed in pancreatic samples obtained at autopsy. The possibility to noninvasively quantify β -cell mass *in vivo* would provide important biological insights and facilitate aspects of diagnosis and therapy, including follow-up of islet cell transplantation. Moreover, the availability of a noninvasive tool to quantify the extent and severity of pancreatic insulinitis could be useful for understanding the natural history of human insulin-dependent (type 1) diabetes mellitus, to early diagnose children at risk to develop overt diabetes, and to select patients to be treated with immunotherapies aimed at blocking the insulinitis and monitoring the efficacy of these therapies. In this review, we outline the imaging techniques currently available for *in vivo*, noninvasive detection of β -cell mass and insulinitis. These imaging techniques include magnetic resonance imaging, ultrasound, computed tomography, bioluminescence and fluorescence imaging, and the nuclear medicine techniques positron emission tomography and single-photon emission computed tomography. Several approaches and radiopharmaceuticals for imaging β -cells and lymphocytic insulinitis are reviewed in detail. (*Endocrine Reviews* 33: 892–919, 2012)

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I. Introduction

The pancreas is a small elongated organ nestled deeply in the retroperitoneum between the duodenum, stomach, and spleen. The majority of the pancreas is made

Abbreviations: AADC, Amino acid decarboxylase; BCM, β -cell mass; BLI, bioluminescence imaging; cMORF, complementary MORF; CNS, central nervous system; CT, computed tomography; L-DOPA, L-3,4-dihydroxyphenylalanine; DTBZ, dihydrotetabenazine; DTZ, dithizone; FDG, fluorodeoxyglucose; GFP, green fluorescent protein; GLP-1, glucagon-like peptide receptor subtype 1; GLP-1R, GLP-1 receptor; 5-HTP, 5-hydroxytryptophan; ID, injected dose; MHC, major histocompatibility complex; MIP, mouse insulin promoter; MNP, magnetic nanoparticles; MORF, morpholino oligomer; MRI, magnetic resonance imaging; NOD, nonobese diabetic; PET, positron emission tomography; SPECT, single-photon emission CT; SPIO, superparamagnetic iron oxide;sstr2, somatostatin receptor type 2; STZ, streptozotocin; SUR, sulfonylurea receptor; T1D, insulin-dependent (type 1) diabetes mellitus; THI, tissue harmonic imaging; US, ultrasound; VMAT, vesicular monoamine transporters type 2.

up of exocrine cells. Dispersed within the normal adult human exocrine pancreatic tissue, there are approximately 1 million distinct microanatomical structures with proper portal-like vasculature and innervation. These structures are known as islets of Langerhans and account for less than 0.005% of the adult body weight. Endocrine α -, β -, δ -, and PP cells are situated within these islets. The β -cells are the insulin-producing cells and account for approximately 70–80% of the cells in the islets of Langerhans.

In addition, a nonexocrine and nonendocrine component is present that is made up of endothelial cells, parasympathetic, sympathetic, and sensory nerve cells, and cells of hematopoietic origin (e.g. monocytes and dendritic cells).

Insulin-dependent (type 1) diabetes mellitus (T1D) is a metabolic disease with complex multifactorial etiology and a poorly understood pathogenesis. Genetic and environmental factors may cause an autoimmune reaction against pancreatic β -cells. Indirect evidence for autoimmunity in human T1D relies on the detection of insulinitis in autopsy samples (Table 1), on the presence of anti-islet-cell antibodies, on T-cell response to β -cell antigens, on the association of diabetes with a restricted set of class II major histocompatibility complex (MHC) alleles, and on the frequent association with other autoimmune disorders such as celiac disease, thyroiditis, vitiligo, and rheumatoid arthritis (1–3).

Examination of islet tissue obtained from pancreatic biopsies of patients with recent onset T1D confirmed the presence of insulinitis in most, but not all, patients. The presence of infiltrating CD4⁺ and CD8⁺ T lymphocytes, B lymphocytes, and macrophages suggests that an inflammatory process has a role in the destruction of the β -cells (4).

The generally accepted model of the natural history of T1D suggests several distinct stages starting with a genetic susceptibility, then autoimmunity without clinical disease, and finally clinical diabetes (1). Nevertheless, other subtypes of T1D have been hypothesized, including the fulminant T1D, which is accompanied by rapid massive destruction of β -cells probably as a consequence of a viral infection (5, 6).

Most of the pathogenetic hypotheses rely on studies performed in two animal models of spontaneous diabetes: BB/W rats (7–10) and nonobese diabetic (NOD) mice (11–14).

In the autoimmune type of T1D, the appearance of autoantibodies is the first sign and precedes several months or years of β -cell destruction, followed by the loss of the first-phase insulin response to the iv glucose tolerance test. The progression to overt diabetes resulting in

significant β -cell destruction is triggered by the development of a more aggressive T-cell infiltration and a change in the T-helper lymphocyte 1, T-helper lymphocyte-2 (Th1/Th2) balance toward a more proinflammatory state (Table 1) (4). When clinical symptoms start, the autoimmune process is already markedly advanced. The β -cell destruction rate, however, is variable, being generally more rapid in children than in adults (15). The exact amount of living and functional β -cells at the onset of clinical symptoms is still unknown because of the lack of an accurate method to quantify functional β -cell mass (BCM) in humans (16). However, some reports suggest that at the time of diagnosis, as much as 60–80% of the BCM can already be dead or dysfunctional.

It therefore emerges that: 1) the availability of a non-invasive tool to quantify the extent and the severity of pancreatic insulinitis could be useful to understand the relationship between the autoimmune phenomena and the progression toward disease onset (17), for early diagnosis of children at risk to develop overt T1D and to select patients to be treated with immune therapies aimed at blocking the insulinitis process, and finally, to monitor the efficacy of these therapies; and 2) the possibility to quantify BCM changes during the early progression of diabetes would also provide important biological insights and facilitate aspects of diagnosis and therapy, including islet cell transplantation (18).

Because of the high clinical relevance of studying insulinitis and BCM *in vivo*, a lot of effort has been put into this research field. Despite that, the ideal imaging tool has not been developed yet, which is mainly due to three problems: 1) the pancreas is not easily accessible by biopsy, thus hampering histological approaches; 2) the islets of Langerhans represent only 2–3% of pancreatic tissue and are spread throughout the pancreatic parenchyma, although more abundant in the pancreatic tail (19, 20); and 3) insulinitis is a chronic asymptomatic process of unknown extent in the prediabetes stage in humans.

When suitable noninvasive imaging techniques become available, high-risk individuals could be monitored before onset of diabetes and over the course of their disease to determine the natural history of the disease and the response to therapy (18).

Structural (anatomical) imaging techniques [magnetic resonance imaging (MRI) and computed tomography (CT)] using specific contrast agents could theoretically achieve the resolution needed to image BCM and insulinitis (21). However, until now, it has not been possible to precisely quantify BCM and insulinitis with these diagnostic imaging techniques. The major problems are: 1) the small size of the islet and the scattered location in the whole pancreas; 2) the small inflammatory lesion during the in-

TABLE 1. Presence of insulinitis and β -cells at T1D onset

Patients (n)	Sex (n)	Age at diagnosis (yr)	Duration of disease	Insulinitis	Other markers	Remaining β -cells	Ref.
4	M (1), F (1) M (1) M (1)	<1 <10 17	1 wk <1 wk <3 wk	+		?	186
				4/4 (100%)			
21	M (2), F (1) M (1) M (2), F (2) M (2), F (1) F (2) M (1), F (3) M (1) M (2), F (1)	<1 >1 ≤ 3 6–13 15 16 17 20–30	<15 d 3 months ≤ 2 months ≤ 1 month ≤ 3 months 4 d 1 d <14 d	+		?	149
				11/21 (52%)			
11	M (2), F (1) F (1) F (1) F (1) F (1) M (3) M (1)	<3 <10 11 <15 17 25–30 31	≤ 4 wk <8 wk ≤ 8 wk 1 wk ≤ 3 wk 1 wk 1 wk	+		+	187
						Degranulated	
				6/11 (54%)			
9	M (2) M (2) F (1) F (1) M (1) M (1) F (1)	2 3 4 5 8 11 12	<8 wk ≤ 4 wk 6 wk 12 wk 8 wk 2 wk 3 wk	+		+	188
				8/9 (89%)		9/9 (100%)	
1	F (1)	12	1 month	+	HLA-I hyper, CD8 ⁺	+	147
				1/1 (100%)			
47	M (11), F (15) M (5), F (8) M (4), F (4)	≤ 10 >10–15 16–19	<9 months <6 months <6 months	+	HLA-I hyper, HLA-DR	+	147, 148
				47/47 (100%)		47/47 (100%)	
18	M (3), F (3) M (1) M (2), F (1) M (2) F (2) M (1) M (3)	>3 5 6–8 10–11 12–14 16 17–21	≤ 3 months	+		?	147, 189 ^a
				19/19 (100%)			
1	M (1)	22	2 d	+	Increase of small lymphocytes and decrease of eosinophils	–/+	190
				1/1 (50%)			
7	M (4) F (3)	>24	<4 months	–	HLA-DR hyper	4/7 (57%)	191 ^a
88	?	1–37	<2 wk	+		+	192
				88/88 (100%)		84/88 (95%)	
18	M (11) F (7)	>18	<7 wk	+	>CD8 ⁺ , <CD4 ⁺ , HLA-I hyper	–/+	193 ^a
				8/18 (44%)			
1	M (1)	19	<1 wk	+	HLA-I hyper, >CD8 ⁺	?	194
				1/1 (100%)			
2	F (1) M (1)	<1 3	<1 month <1 wk	+		?	195
				2/2 (100%)			
17	M (11) F (6)	>18	<1 month	+	HLA-I hyper	?	196 ^a
				8/17 (47%)			

(Continued)

TABLE 1. Continued

Patients (n)	Sex (n)	Age at diagnosis (yr)	Duration of disease	Insulinitis	Other markers	Remaining β -cells	Ref.
1	F (1)	65	<1 yr	+	DR4, >CD4 ⁺ , <CD8 ⁺	?	197 ^a
29	?	Mean 28 yr	3 months	1/1 (100%)	CD8 ⁺ , aberrant HLA-I	?	198 ^a
2	?	Children	Acute onset	17/29 (58%)	CD45R ⁺ , CD56 ⁺ , CD3 ⁺	?	199
				2/2 (100%)			
Summary 277	M + F	0–37 (one of 65 yr)	0–1 yr	223/270 (82.6%)	HLA-I, HLA-II, CD3, CD4, CD8, CD45, CD56	144/151 (95.4%)	17 papers

F, Female; M, male; HLA-I, human leukocyte antigen class I; HLA-II, human leukocyte antigen class II; HLA-DR, human leukocyte antigen class DR; CD, cluster differentiation antigens; hyper, hyper-expression.

^a Detected by biopsy.

sulitis process; and 3) the lack of information about β -cell functionality and immune cell activity.

However, recent advances in noninvasive imaging techniques such as molecular studies applied to MRI, positron emission tomography (PET), and optical imaging indicate that there could be a role for diagnostic imaging in the evaluation of β -cell number, mass, and function and lymphocyte infiltration/inflammatory activity in T1D (22). With the current functional imaging methodology, the single islet cannot be spatially resolved. Therefore, the islet cells or the islet-infiltrating lymphocytes must be chemically resolved with a specific probe that has high specificity for target combined with a low background signal. Thus, the target should retain the labeled probe at least 1000-fold more avidly than the surrounding exocrine cells, resulting in sufficient contrast between the exocrine and endocrine pancreas (23). In addition, the probe should be metabolically stable in circulation and show suitable pharmacokinetic properties. Combined structural and functional imaging might be a suitable approach to overcome the weaknesses of each individual technique. There is a vast amount of literature concerning BCM and insulinitis imaging. In this review, we will outline the imaging techniques currently available for *in vivo*, noninvasive investigation of BCM and insulinitis.

II. Radiological Techniques for Imaging BCM and Insulinitis

Considering its anatomic location, the propensity for autolytic damage, and the distribution of islets within the exocrine tissue, pancreatic biopsies to quantify the BCM and/or detect the insulinitis are not easily performed. Diagnostic imaging of the endocrine pancreas is also problematic mainly for the difficulties to detect islets of Langerhans in the pancreatic parenchyma. The radiological modalities

for pancreatic imaging include MRI, ultrasound (US), and CT (21).

A. Magnetic resonance imaging

MRI is a noninvasive approach that offers high spatial resolution, especially in comparison with nuclear medicine techniques and high intrinsic contrast *in vivo*. It is a promising technique for islet imaging because it can target possible β -cell-specific components, using various mechanisms for contrast enhancement and achieving high-resolution images (24) with prolonged visualization of the labeled cells. In fact, the MRI contrast agents do not have the problems related to the use of radionuclides (decay and short half-life) and may allow imaging over a long period of time. Although it remains to be determined whether the sensitivity and applicability of MRI are sufficient for human clinical applications, MRI has showed very promising results in animal models in the study of insulinitis, pancreatic BCM, and islet transplantation.

Early biomarkers of pancreatic insulinitis that can be investigated by MRI are the islet microvascular dysfunction and the alteration in vascular volume, flow, and permeability, as described in several models of T1D.

Using a paramagnetic contrast agent, gadolinium diethylene triamine pentaacetic acid-fluorinated (PCG-GdDTPA-F), with high plasma half-life, detectable on T1-weighted MR acquisition, it was possible to evaluate *in vivo* vascular changes in a streptozotocin-induced mouse model of T1D, demonstrating a significantly higher accumulation of gadolinium diethylene triamine pentaacetic acid-fluorinated in the pancreas of diabetic animals compared with normal animals (25).

A specific magnetic nanoparticle (MNP) [monocrystalline iron oxide nanoparticles (MION-47)] for MRI has been proposed to identify and quantify the vascular volume and permeability changes associated with inflammation of the pancreas during the development of autoim-

mune diabetes in rodents (NOD/Lt, E α 16/NOD, and BDC2.5/NOD mice). This method relies on the measurement of the microvascular changes associated with inflammation, and it is not hampered by the variations in MHC alleles or autoreactive T-cell specificities that limit other experimental approaches (26). The pancreatic inflammation can also be detected using long-circulating phagotropic nanoparticles that extravasate from the leaky vessels into the surrounding tissue and are engulfed by infiltrating inflammatory cells, particularly macrophages. Because MNP are superparamagnetic and thus negative T2-weighted contrast agents, signal changes of pancreatic parenchyma (reduction of signal intensity) on T2-weighted scans as a surrogate for vascular leak/macrophage uptake were used. Improvement of this approach has been done using nanoparticles with a superparamagnetic iron oxide (SPIO) core coated with dextran (27). This modification can reduce immunogenicity of the particles and increase blood resident time. A potential application of this imaging technique is monitoring acute changes in pancreatic inflammation in patients who have previously undergone intervention trials to treat or prevent T1D (26).

The second approach to monitor changes during the progression of diabetes is to follow *ex vivo* lymphocytic accumulation in the pancreas. Tracking labeled autoreactive T lymphocytes was used to detect *in vivo* chronic mononuclear cell infiltration into the pancreas during the prediabetic phase. This approach is based on the intracellular labeling of lymphocytes with superparamagnetic contrast agents (dextran magnetic particles), which are taken up by the cells by membrane diffusion. To increase the uptake of nanoparticles inside the cells, the nanoparticles can be modified to increase their hydrophobicity or can be encapsulated into liposomes (28).

Dextran-coated SPIO nanoparticles coupled with peptides encoding for the membrane translocation signal of HIV-1 (CLIO-Tat) were used to label lymphocytes with an efficacy of internalization that was 100 times higher than normal particles. After 30-min incubation, the nanoparticles have not only entered the cell but were also bound to the nucleus and nucleolus (nuclear accumulation) (29, 30).

Therefore, CD8⁺ lymphocytes labeled with these nanoparticles were used to track the immune cells in animal models of T1D. After adoptive transfer of nanoparticle-labeled T cells into the recipient animal (NOD, Scid mice), it was possible to visualize the labeled T lymphocytes infiltrating the pancreas by the signal intensity decrease in the pancreatic parenchyma on T2-weighted images. The decrease of pancreatic parenchyma signal on T2-weighted images due to lymphocytic infiltration was not present in control animals without insulinitis. To improve these re-

sults, specific subpopulations of diabetogenic lymphocytes were isolated from transgenic NOD mice and loaded with CLIO-NRP-V7-coated superparamagnetic nanoparticles. These particles specifically bind to T-cell receptor α on NRP-V7-reactive CD8⁺ T cells. The labeled lymphocytes allowed the visualization in real time of the progressive accumulation of NRP-V7-reactive CD8⁺ T cells into the pancreas of NOD mice (31). The target of this lymphocyte subpopulation is the islet glucose-6-phosphatase catalytic subunit-related protein, and consequently, this selected population of lymphocytes should selectively migrate into the pancreas. Because islet glucose-6-phosphatase catalytic subunit-related protein is also present in the human islets, this approach might allow translation of results to human imaging.

There is only one recent report in humans that describes the use of MNP as a contrast agent for MRI in T1D to image insulinitis. Gaglia *et al.* (32) evaluated 10 patients with T1D and 12 nondiabetic controls. They used MRI MNP previously validated in mouse models, ferumoxtran-10, which has a dextran coating and size similar to those used in animal experiments. These particles are taken up by macrophages and do not induce cell activation or proinflammatory cytokine or superoxide anion production. MRI can detect MNP that migrate from the leaky vessels into the surrounding tissue (extravasation) and are phagocytized by inflammatory cells, particularly by macrophages. MNP are negative T2-weighted contrast agents because they induce changes in T2 signal of pancreatic parenchyma (reduction of signal intensity) as a surrogate parameter for measuring vascular leak and macrophage content. The results of the study by Gaglia *et al.* (32) showed that T1D patients at the time of diagnosis already have a reduced pancreatic volume compared with controls and showed heterogeneity in MNP accumulation. Although not testable in this small study, one could speculate that such differences reflect heterogeneity in the insulinitis process itself. Therefore, this technique requires further development to determine its sensitivity and specificity (32).

Most MRI studies have been performed for imaging BCM and transplanted islets in animal models of T1D.

Gimi *et al.* (33) used manganese (Mn), a contrast agent for MRI with T1-relaxation properties, as a tool to image *in vitro* β -cell functionality in cell cultures of isolated islets. Similar to calcium, extracellular Mn was taken up by glucose-activated β -cells, resulting in a 200% increase in MRI contrast enhancement *vs.* nonactivated cells. This scientific work can be considered the first molecular MRI study for β -cell imaging. After this study, several other approaches were tested.

Nevertheless, most MRI studies have evaluated the efficacy of islet transplantation to replace β -cell loss in T1D. MRI can monitor islet transplantation and detect causes of islet failure.

Although islet transplantation is a promising therapeutic approach, substantial islet loss can occur due to allo-rejection, autoimmune attack, glucose toxicity, mechanical stress, and microvascular disruption of the islets during their isolation from the donor and their transplantation. The strategies to visualize with MRI the phenomena relevant to islet transplantation involve labeling of isolated islets with a contrast agent and their transplantation into the recipient. In the first experience (34), purified pancreatic islets labeled with a commercially available SPIO (Bayer Schering Pharma, Leverkusen, Germany) were transplanted through the portal vein into the liver of experimental rats. The labeled pancreatic islets were clearly visualized in the liver of both diabetic and healthy rats as hypointense areas on T2-weighted MR images for a long period.

The labeling procedure facilitates detection and monitoring of the transplanted islets, but we should make the following considerations: 1) labeled nonfunctioning islets cannot be distinguished from functioning ones, and therefore, transplantation of labeled islets does not directly provide any information on the function, short-term viability, or absolute mass of β -cells (35); 2) some phenomena, such as gradual islet mass replacement by amyloid, cannot be detected by the described methods unless the agent used to label pancreatic islets is specifically designed to label β -cells; and 3) these methods were mainly used in small animals, and there is just one report concerning its application in humans by Toso *et al.* (36).

This study by Toso *et al.* (36) demonstrated the feasibility and safety of MRI-based islet graft monitoring in clinical practice by MRI of transplanted islets labeled with iron nanoparticles. Here, isolated islets were cultured with SPIO nanoparticles for 24–72 h to obtain cell uptake via random endocytosis.

Kriz *et al.* (37) investigated MRI of labeled islets transplanted in the liver of diabetic rats and concluded that iron-labeled pancreatic islets can be used for the detection of the decreasing relative islet mass due to rejection. Nevertheless, this methodology could not be easily applied to quantitative studies, and another approach was tested: the use of automatic quantitative ultrashort echo time imaging protocol. This protocol gives a positive enhanced contrast to labeled cells, suppressing liver and small vessel signal, thus allowing a precise *in vivo* quantification of iron-labeled pancreatic islets transplanted into the liver (38).

Heparinized SPIO nanoparticles (heparin-SPIO) were also synthesized for chemical labeling of β -cell surface as

proposed by Jung *et al.* (39). Compared with typical cellular labeling with SPIO via endocytosis, surface labeling with heparin-SPIO was shown to be more stable and improved in animal models the hypointensity of transplanted islets on T2-weighted MR images due to surface modification of each islet.

The MRI detection of islet labeled by iron oxide-based contrast agents and transplanted into the rat liver parenchyma could be significantly improved using the iv administration of a suitable contrast agent, gadolinium-benzoyloxypropionictetra-acetate (Gd-BOPTA), before MRI as proposed by Herynek *et al.* (40).

On the labeling of endothelial progenitors of islet cells, there is only one report focused on the use of contrast agent gadolinium(phen)HDO3A (Gd-HDO3A), a paramagnetic contrast agent that is visualized on T1-weighted images. With this approach, authors demonstrated *in vitro* and *in vivo* the visualization of islet transplanted mouse islets under the mouse kidney capsule and xenotransplanted human islets in the mouse liver (41).

B. Ultrasonography

US imaging is one of the most used methods for the clinical and diagnostic evaluation of the pancreas due to its safety (no ionizing radiation exposure for the patient), high availability in clinical settings, and relative ease of use.

The applications of US to medical imaging cover different diagnostic aspects: the anatomical structure of the pathological organs and their functionality by dynamic visualization of the organ blood flow.

Several anatomical and structural changes that occur in the pancreas during T1D can be used for diagnosis of diabetes mellitus, although US is rarely used in daily routine for this purpose.

The lack of visualization and quantification of the islets of Langerhans is a major drawback of US that can detect pathological changes only of the entire organ caused by atrophy of the pancreatic islets and by several other alterations. Its use in T1D is therefore limited.

In a series of studies, the pancreas in T1D patients was shown to be smaller than in the healthy controls, and one of the causes was found to be the atrophy of islets of Langerhans. In T1D patients, the thickness of head, body, and tail is 1.9 ± 0.3 , 0.9 ± 0.2 , and 1.4 ± 0.2 cm, respectively, whereas in age-matched normal subjects, the thickness is 2.4 ± 0.4 , 1.1 ± 0.3 , and 1.8 ± 0.4 cm, respectively. The size of the pancreas is not correlated with the age of the patient, but the decrease of the pancreatic volume is more evident during the first year of T1D, involving particularly the head/body and tail/body (42–44).

Even if US has been proved to be useful in detecting the morphological change in the pancreas of diabetic patients,

it is nowadays not possible to visualize any change before the clinical onset of the diseases.

Recently, the possibility to use US as a noninvasive method for the detection of intraportal islet transplantation was investigated. The main finding was the induction of focal fatty infiltration, steatosis of the liver, which is easily detectable with US (45).

Enhancement of the signal-to-noise ratio was better achieved by tissue harmonic imaging (THI), a new technology that improves the image quality compared with conventional US (46). THI is based on the phenomenon of nonlinear distortion of an acoustic signal through the body. Harmonic wave frequencies are higher-integer multiples of the transmitted frequency, much like the overtones of a musical note. Current technology uses only the second harmonic for US imaging. The processed image is formed with use of the harmonic-frequency-band width in the received signal after the transmitted frequency spectrum is filtered out. THI provides better axial resolution by using high frequencies without compromising depth penetration. It is particularly useful for imaging cystic lesions and those lesions containing highly reflective tissues such as fat, calcium, or air. Harmonic imaging is particularly helpful in obese patients because of the reduction of the defocusing effect of the body wall. Therefore, in patients with a body mass index of 30 kg/m² or more, THI is clearly better than conventional US, allowing better detection of all abdominal organs, including the pancreas.

Promising results were obtained in the postoperative monitoring of kidney/pancreas transplantation for the evaluation of pancreas graft rejection. Better detection and demarcation of pancreas graft was found (91 *vs.* 78% of conventional US in T1D patients) (47). The limit of this technique is its low accuracy.

C. Computed tomography

CT is another radiological imaging technique used to visualize anatomical modifications of a retroperitoneal organs, such as the pancreas, and is based on the differential attenuation of x-ray by different tissues (48, 49).

The islets of Langerhans are small, almost homogeneously distributed throughout the exocrine pancreas and have a similar density as the rest of the pancreas. Therefore, it has not been possible yet to discriminate the islet from the exocrine pancreas using the available CT scanners. The recent availability of 256 and 512 multidetector high-resolution CT scanners opens new possibilities to the study of the pancreas, although the slight modifications of the pancreatic parenchyma in terms of alterations in blood vessel density and lymphocytic infiltration during diabetes are probably too small to effectively discriminate the healthy from the pathological pancreas.

Advances in CT detector technology can now take advantage of the polychromatic nature of the x-ray spectrum when creating CT images (spectral CT imaging). In dual-energy CT, the simultaneous use of two different-energy x-rays allows the differentiation of tissues on the basis of their energy-related attenuation characteristics. Spectral CT can therefore facilitate the discrimination of tissues, making it easier to differentiate between materials, such as tissue containing calcium and iodine that can appear similar on traditional monochromatic CT techniques. Moreover, this technique could reduce x-ray radiation dose and is hypothesized to increase imaging sensitivity to contrast agents. Improving the sensitivity of CT to low amounts of contrast agent may enable the use of novel contrast agents, allowing CT to provide molecular and physiological information (50) as well as to evaluate pancreatic perfusion.

CT has also been used to visualize the modifications that occur after pancreas or kidney-pancreas transplantation for treatment of T1D. After transplantation, there are usually modifications in the surrounding tissue, such as fat infiltration (steatosis), altered vessel organization, thrombosis, and acute lymphocytic infiltration of lymphocytes (51). These changes reflect strong modification of the density and vascularity of the organ that can be detected by contrast-enhanced CT.

In conclusion, nowadays, CT is not suitable to visualize BCM and insulinitis during the natural history of diabetes, but this technique holds good promise for the future.

D. Concluding remarks on radiological techniques for imaging BCM and insulinitis

MRI, due its high spatial resolution, is the most promising radiological technique for imaging BCM and insulinitis. There are new nanoparticles that can be used to visualize BCM and insulinitis, thus allowing the identification of islet pathologies. It also offers the important advantage over CT of avoiding radiation exposure and being the technique of choice to follow up children at risk for diabetes.

US has no major role for β -cell and insulinitis imaging. CT, using new technologies, could be a good alternative to image morphological alterations after pancreatic transplantation, but it cannot be used for imaging insulinitis.

III. Optical Imaging Techniques for Imaging BCM and Insulinitis

Optical imaging (bioluminescence and fluorescence imaging) was developed as a new strategy to study molecular and biological events, such as cell trafficking, apoptosis, protein-protein interactions, *etc.* Optical imaging is based on the use of labeled molecules that emit photons with a

specific wavelength that is detectable outside the body with sophisticated instruments.

Recently, optical imaging was applied to study T1D because it can potentially give quantitative and spatial information about islets in the pancreas.

A. Bioluminescence

Bioluminescence imaging (BLI) has been investigated as a noninvasive method to quantify and visualize BCM. The strategy was to develop a transgenic construct with a specific promoter that regulates the expression of a reporter gene (*i.e.* luciferase enzyme) in the target cells. For diabetes research, the reporter gene was placed under control of the mouse insulin promoter (MIP) (52). Consequently, the expression of the reporter gene was induced only in pancreatic β -cells of transgenic mice that produced insulin and contained this construct. In β -cells that expressed the reporter gene, the enzymatic reaction between the reporter enzyme (luciferase) and a substrate (luciferin) produced photons that can be captured by a charge-coupled detector camera (53). The bioluminescent signal emitted by the cells expressing the transgene is specific for β -cells, no signal is observed in other tissues, and an excellent correlation was found between the bioluminescent signal and the number of islets ($r^2 = 0.986$). Moreover, the activity of luciferase increased 2.5-fold in hyperglycemic conditions, indicating that bioluminescence is also correlated with the metabolic status of the cells. In addition, no auto-bioluminescence occurred in normal tissues, meaning that a high target-to-background ratio can be obtained. The good correlation between the luciferase expression and cellular condition makes the BLI a good candidate for β -cell imaging. β -Cells transfected with luciferase under control of MIP were successfully applied to follow up graft rejection after islet transplantation.

In case of syngeneic transplantation of 250 tagged islets in FVB/Nj mice, the signal decreased by 46% in the first 2 wk after transplantation, but after this period, the signal remained constant for 160 d. After allogeneic islet transplantation in BALB/c streptozotocin-induced diabetic mice, the bioluminescent signal decreased by 55% at d 19 after the islet inoculation. Immunohistochemistry confirmed that CD3⁺ T cells were responsible for the graft rejection after transplantation.

The sensitivity of this BLI technique was investigated also in animals transplanted with 10–150 tagged islets in different parts of the body (liver, subcapsular renal space, ip, and sc). The strongest signal was obtained when islets were inoculated sc. These results underline the main disadvantage of optical imaging, attenuation of photons by tissue, which hampers the detection of signals from deeply located tissues and organs (54). Consequently, this tech-

nique is applicable only to small animals or superficial tissues.

B. Fluorescence imaging

Fluorescence imaging is based on the use of specific molecules labeled with a fluorophore. The migration of the labeled molecules in tissues is subsequently monitored using a dedicated camera or microscope (55).

The fluorescent label must be irradiated with an external light source with an appropriate wavelength to bring the fluorophore to an excited state. Subsequently, the fluorophore will return to its ground state and emit light with a longer wavelength. The most common fluorophore is the green fluorescent protein (GFP) that emits a green light with a wavelength of 509 nm (56, 57).

There is a growing list of other fluorophore such as a Cy5.5, quantum dots, and near-infrared probes. Fluorescent probes have been used in microscopy as a tag for the subcellular localization of biomolecules under normal and pathological conditions. Recently, the possibility to use these fluorescent molecules to visualize different cell subtypes *in vivo* in animal models was also explored (58–60).

The GFP gene was put under control of a β -cell-specific promoter, such as MIP, to induce the tissue-specific expression of the fluorescent probe in β -cell cultures (61–63).

In 2003, Hara *et al.* (64) generated transgenic mice with stable and specific expression of GFP in β -cells. Preliminary data indicated that it is possible to quantify BCM *ex vivo* using fluorescence-activated cell sorter analysis. In addition, this strategy was applied to isolated β -cells by a cell sorter to obtain a pure population of β -cells expressing GFP for *in vitro* studies.

This genetically engineered mouse model could be used to study β -cell development from the embryonic stage to the adult stage. In addition, this model was suitable to study the three-dimensional distribution of islets in the pancreas and to quantify the BCM during the different stages of diabetes (64).

Major limitations of fluorescence imaging are the autofluorescence of endogenous biomolecules, such as hemoglobin and collagen, and the light absorption by surrounding tissues, causing an increase in the background signal and a reduction in the target signal, respectively. Near-infrared fluorophores are more promising than GFP because they suffer less from autofluorescence or absorption by tissues.

Recently, Reiner *et al.* (65) published a study about the measurement of BCM using exendin-4 conjugated with the near-infrared fluorophore (VT750) that was produced via copper-catalyzed click chemistry. This fluorescent probe (E4_{X12}-VT750) is able to selectively bind to the glucagon-like peptide receptor subtype 1 (GLP-1), which

is expressed on β -cells. Intravital microscopy, histology, and endoscopy proved that there was an excellent correlation between islet area and islet size with the VT750 fluorescent signal ($R^2 = 0.944$ and 0.992 , respectively). A good correlation between BCM estimated with immunostaining for insulin and the VT750 fluorescent signal ($R^2 = 0.854$) was also observed.

Optical techniques are new methods to visualize BCM in research settings, but these techniques cannot be applied in humans because of the limited penetration of light through tissues.

C. Concluding remarks on optical imaging techniques for imaging BCM and insulinitis

Optical imaging offers excellent possibilities for imaging both BCM and insulinitis. This has been well documented in animal models, particularly for time-course studies.

The application of these techniques in humans is hampered by the deep location of the pancreas in the body and by the difficulty of detecting fluorescent light with the currently available technology.

IV. Nuclear Medicine Techniques for BCM Imaging

Several approaches have been used to image β -cells with radioactive probes, as summarized in Table 2 and Fig. 1 and described below in detail.

A. Imaging β -cell secretory granules (Zn^{2+} -mediated secretory vesicle formation)

Zn^{2+} plays a critical role in the biosynthesis, transport, maturation through the Golgi apparatus, and secretion of insulin. Zinc ions are essential for packaging insulin into the secretory vesicles by formation of hexamers of insulin, which are ready to be released upon glucose demand (66, 67).

Dithizone (DTZ) is currently used as a dye for histological examination and to monitor cell survival *in vitro*.

Latif *et al.* (68) have studied the mechanism of staining β -cells with DTZ. DTZ permeates the cell membrane and complexes with Zn^{2+} in the secretory vesicles and remains stored in the granules of pancreatic β -cells. In 1988, Latif and co-workers (68) demonstrated that DTZ is able to stain specifically pancreatic islets.

Later, Fiedor *et al.* (69) used an iodine DTZ analog to radiolabel *ex vivo* the islets of Langerhans isolated from healthy Lewis rats. These DTZ-labeled islets were syngeneically transplanted in the renal subcapsular space of diabetic recipients and were shown to have a long-term sur-

vival. Because DTZ is a potentially toxic compound, the correlation between the doses injected, the time from injection, and the toxicity was studied *in vivo*. At low concentrations and at short intervals after DTZ administration, no side effects of this radiopharmaceutical were found in target cells. DTZ administration does not interfere with the normal endocrine function of β -cells (69–71). Encouraged by these results, other studies were performed that explored the possibility to use DTZ labeled with a radioisotope for *in vivo* monitoring of the viability of the pancreatic islets.

The most frequently used isotope for labeling DTZ is ^{131}I , although labeling yields were poor. In addition, [^{131}I]DTZ is an unstable compound in biological fluids. To stabilize the radiopharmaceutical, Garnuszek *et al.* (72) have developed a new radioiodination procedure that produced a more stable radiolabeled compound: 2–2'-dicarboxy dithizone- ^{131}I histamine. It possessed the ability to bind Zn^{2+} , creating a stable complex that is trapped in the secretory vesicles of β -cells. Moreover, the *in vivo* tests in Wistar rats showed a high pancreatic uptake [$12.41 \pm 2.33\%$ of the injected dose (ID)/g tissue] after injection in the splenic artery with blockade of the liver artery. However, when the radiolabeled DTZ was injected into the tail vein of the rats (thus allowing liver metabolism of DTZ) the uptake in the pancreas was very low ($0.81 \pm 0.27\%$ ID/g) (72).

Recently, a radioactive fluorinated DTZ analog was synthesized for PET imaging. [^{18}F]DTZ was tested in *in vitro* and *in vivo* studies to evaluate specific uptake and retention. Three different cell types have been used *in vitro*: isolated β -cells from rats, an exocrine pancreatic cell line, and an endocrine cell line. The data suggest that the uptake was nonspecific, because radiopharmaceutical binds in both the endocrine and the exocrine cell lines. Moreover, the retention of the radiopharmaceutical in the pancreas was low *in vivo* and was comparable to that in other organs. Significant uptake was also found in the spleen, stomach, lungs, heart, and fat, resulting in a low signal-to-background ratio (73).

Taken together, these results indicate that uptake of radiolabeled DTZ is low and not exclusive for β -cells. Moreover, the toxicity of this compound may be another hurdle for its use in patients (74). Therefore, DTZ is not a good candidate as a probe for noninvasive imaging of β -cells.

B. Imaging ATP-sensitive potassium channels

ATP-sensitive potassium channels are present on the surface of many kinds of neuroendocrine cells including β -cells (75).

ATP-sensitive potassium channels on β -cells are composed of two different subunits. The first one is a small

TABLE 2. Radiopharmaceuticals used for β -cell imaging

Radiopharmaceutical	Isotope	Type of study	Target	Pros	Cons	Ref.
DTZ	^{131}I	Animal studies	β -cell secretory granules	High pancreas accumulation	Isotope, low LE, unstable	69, 72
DTZ	^{18}F	<i>In vitro</i> and animal studies	β -cell secretory granules	High uptake and specificity for β -cells	Low retention	73
Repaglinide	^{11}C	<i>In vitro</i> studies	β -cell SUR1	High affinity for SUR1	Low LE, lack of quantitative data	82
Repaglinide	^{18}F	<i>In vitro</i> studies	β -cell SUR1	High affinity for SUR1 (lower as compared with [^{11}C]repaglinide)	Low LE, low accumulation in β -cells	83
Glibenclamide (glyburide)	^3H	<i>In vitro</i> studies	β -cell SUR1	High uptake, high specificity for β -cells	Isotope features, low specific activity, low retention	86
Glibenclamide (glyburide)	^{18}F	<i>In vitro</i> and animal studies	β -cell SUR1 and -2		Low uptake <i>in vivo</i> on pancreas, low specificity	87
Naphthylalanine derivatives	^{123}I	<i>In vitro</i> and animal studies	sstr2	High accumulation in the pancreas	Low affinity for the receptor	94, 95
L-DOPA	^3H	<i>In vitro</i> and <i>in vivo</i> studies	AADC	Specific accumulation <i>in vivo</i> on β -cells	No selectivity <i>in vitro</i> for β -cells	103–105
Dopamine	^3H	<i>In vitro</i> studies	AADC	Water solubility, rapid clearance, low plasma protein binding	Moderate selectivity for β -cells	73
Serotonin	^3H	<i>In vitro</i> studies	β -cell secretory granules		Low uptake and retention in β -cells, low specificity	104, 109
5-HTP	^{11}C	<i>In vivo</i> studies	Large amino acid transporter (LAT)	Stored in secretory granules		110, 111
DTBZ	^{11}C	Animal studies/human studies	Ligand for VMAT2	High target-to-background ratio	Short half-life	116, 118
DTBZ	^{18}F	Animal study	Ligand for VMAT2	High pancreatic uptake	Uptake in surrounding organs (high background activity)	115, 120
4-Fluorobenzyltrozamicol	^{18}F	Animal and human (1 patient) studies	Presynaptic vesicular acetylcholine transporter	High uptake	Low specificity for β -cells	123
FDG	^{18}F	<i>In vitro</i> studies	β -cell metabolic pathway		Low specificity for β -cells, low uptake	73, 126
Nicotinamide	^{14}C	<i>In vitro</i> studies	β -cell metabolic pathway	High retention	Low uptake, low β -cell specificity	73
GLP-1	^{125}I	<i>In vitro</i> studies	GLP-1R	High and specific uptake on β -cells	Lysosomal degradation	135
Exendin-3	^{68}Ga	<i>In vivo</i> studies	GLP-1R	High and specific uptake in β -cells	Preliminary data	136
Lys 40 -(Ahx-DTPA)exendin-4	^{111}In	<i>In vitro</i> and animal studies	GLP-1R	High uptake	No specific binding on β -cells	137, 138
Anti-IC2 mouse pancreatic β -cell (IC2 MoAb)	^{111}In	Animal studies	IC2 antigen	β -cell specificity	Slow clearance and uptake, no quantitative data	141, 142
MORF/cMORF	$^{99\text{m}}\text{Tc}$	Animal studies	TAG-T2	High specificity for β -cells	Intestinal uptake	143
MORF/cMORF	^{111}In	Animal studies	TAG-T2	High specificity for β -cells	Preliminary data	143

MoAb, Monoclonal antibody; LE, labeling efficiency.

potassium channel, Kir 6.2, that forms a pore and contains the ATP-binding site. The second is the regulatory sulfonylurea receptor (SUR) with an intracellular Mg nucleotide activation site (76).

In β -cells, only the SUR1 subtype was identified. SUR1 is a large trans-membrane protein belonging to the ATP-binding cassette protein superfamily with two intracellu-

lar nucleotide-binding sites. The complete ATP-sensitive potassium channel is formed by four Kir 6.2 and four SUR1 subunits (77). This channel regulates the insulin release in response to changes in plasma glucose concentration through the integration of signals from different pathways, such as changes in membrane potential and ATP levels (78).

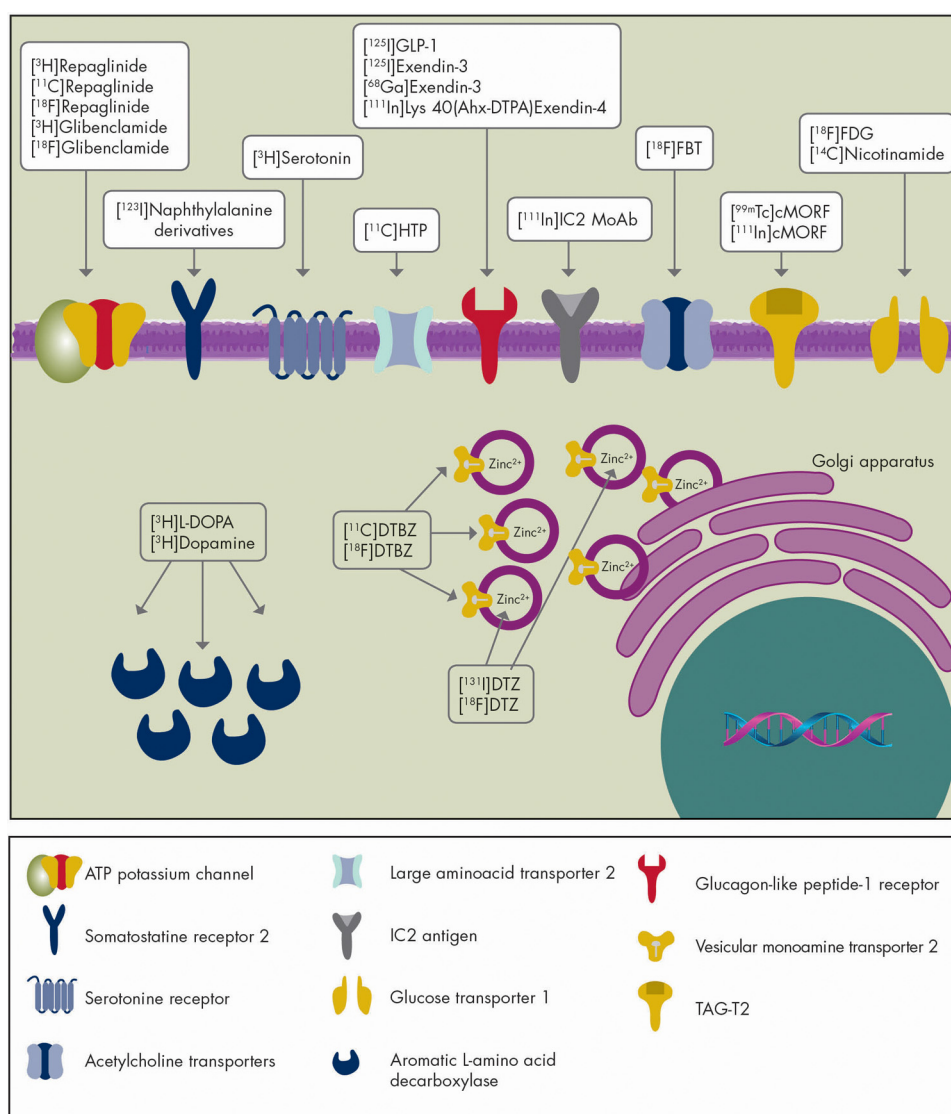
Figure 1.

Figure 1. Schematic structure of a pancreatic β -cell showing relevant receptors that have been targeted by different radiopharmaceuticals for β -cell imaging.

When the glucose concentration in plasma is high, the ATP-sensitive potassium channels are closed, resulting in a membrane depolarization that opens the voltage-sensitive Ca^{2+} channels. The result is an influx of Ca^{2+} into the cell, which stimulates the exocytosis of intracytoplasmic vesicles, in which insulin is stored. In this way, glucose homeostasis is maintained (77). In recent years, several hypoglycemic drugs have been developed that bind to ATP-sensitive potassium channels. Most of the sulfonylurea drugs used for diabetes treatment act at low concentrations by inhibiting the SUR subunit (79). The new generation of sulfonylureas, which specifically target β -cells without strong side effects, can be used as lead compound

for developing PET and single-photon emission CT (SPECT) radiopharmaceuticals.

1. Repaglinide

Repaglinide is a drug that binds to the SUR (80, 81). Repaglinide was labeled with ^{18}F by alkylation with 2- $[^{18}\text{F}]$ fluoroethyl tosylate and with ^{11}C by ^{11}C -methylation. Both radiopharmaceuticals were prepared in high radiochemical yield (20 and 35%, respectively) and had a high radiochemical purity (>98 and >99%, respectively).

For both compounds, binding assays were performed to determine the affinity of the radiopharmaceutical for the SUR. For these experiments, COS-1 cells that express

the human SUR1 on the cell surface were used. Moreover, insulin secretion experiments on rat islets were performed to test the functionality of this compound *in vitro*.

These experiments showed that [^{11}C]repaglinide binds with high affinity and specificity to SUR1. Because the molecular structure of the ^{11}C -labeled radiopharmaceutical is identical to the structure of repaglinide, and its biological activity is also identical to that of the unlabeled drug (82).

[^{18}F]Repaglinide shows lower specific binding than [^{11}C]repaglinide, suggesting that the fluoroethyl group decreases the binding affinity and the biological activity. Despite the lower specific binding of [^{18}F]repaglinide compared with the radiopharmaceutical labeled with ^{11}C , a biodistribution study in rats was performed. The results indicated that 10–30 min after injection, there is a stable accumulation of this radiopharmaceutical in the pancreas, suggesting that [^{18}F]repaglinide has potential for human PET imaging (83).

2. Glibenclamide

Glibenclamide (glyburide) is a second-generation sulfonylurea. Compared with first-generation drugs, it is more efficient at low glucose blood concentrations and has fewer side effects. Glyburide has the same mechanism of action as repaglinide, but it is three to five times more effective (84, 85).

Glyburide was first labeled with tritium for *in vitro* studies to determine the specificity, accumulation, and retention in INS-1 β -cells and PANC-1 exocrine pancreas cells as control. Freshly isolated β -cells from rat islets were also used. The uptake of [^3H]glyburide was high in β -cells, but not specific, because high uptake was also found in exocrine cells, which do not express SUR1. The study also showed that [^3H]glyburide uptake decreased in the presence of albumin, thus making this radiopharmaceutical not suitable for *in vivo* imaging (86).

Despite these disappointing results, [^{18}F]glyburide was synthesized and tested in two β -cell lines with good results. When the cells were preincubated with an antibody against SUR, decreased [^{18}F]glyburide binding was observed, indicating that the binding is receptor mediated. This radiopharmaceutical was not tested in an exocrine cell line, and therefore, its specificity for β -cells was not determined.

Normal and streptomycin-induced diabetic SCID mice were used to determine the usefulness of this radiopharmaceutical *in vivo*. Pancreatic uptake was low, and the background signal was high in both groups of animals. This could be due to the binding of glibenclamide to SUR1 and SUR2 with similar affinity. SUR2 is also expressed in other tissues such as cardiac, smooth, and skeletal muscle,

thus increasing the background signal. Control experiments, where the radiopharmaceutical was administered after injection of an excess of unlabeled glibenclamide, showed that the radiopharmaceutical uptake was not blocked and therefore not specific (87). One of the main problems of glyburide is its high lipophilicity causing high nonspecific binding and plasma proteins.

Recently, Schneider *et al.* (88) have studied a new glyburide-glucose conjugate. Binding affinity studies were performed in COS-1 cells transfected with human SUR1, but the new glycosylated compound showed lower binding affinity than the nonglycosylated drug. However, the glyburide-glucose conjugate has 40% less activity for stimulation of insulin secretion compared with the nonglycosylated compound. Glycosylation also increases the molecule's hydrophobicity and plasma clearance, thus decreasing nonspecific binding and accumulation in liver and intestine. Consequently, an improved signal-to-noise ratio was obtained, which is beneficial for the detection of pancreatic islet cells *in vivo*.

Among the aforementioned radiopharmaceuticals, glyburide-glucose conjugate appears the most useful for β -cell imaging, but further evaluation of the compound is still required.

C. Imaging neurotransmitter targets on β -cells

Somatostatin, dopamine, and serotonin receptors and metabolic enzymes for these neurotransmitters are expressed not only in the central nervous system (CNS) but also in β -cells. As a consequence, these proteins might be potential targets for molecular imaging of BCM (Fig. 2).

1. Somatostatin receptor imaging

The somatostatin receptor type 2 (sstr2) is localized in all pancreatic cell types, but it is most abundant in β -cells. High expression of various subtypes of the receptor was also found in pancreatic tumors (89).

Radiolabeled somatostatin analogs are currently used for diagnosis and staging of neuroendocrine tumors expressing sstr (90). Among the somatostatin analogs that have been radiolabeled are octreotide (91), lanreotide (92), and vapreotide (93), which bind to several sstr subtypes with different affinity. These analogs have been labeled with several radioisotopes, which give them different characteristics in terms of stability and imaging resolution. So far, however, these radiopharmaceuticals have not been applied for imaging BCM.

Amartei *et al.* (94) have synthesized a new iodinated nonpeptide compound based on β -naphthylalanine and *n*-hexanediamine. The radiopharmaceutical shows low affinity for sstr2, but biodistribution data in normal CBA/J mice showed that there is a high accumulation of this ra-

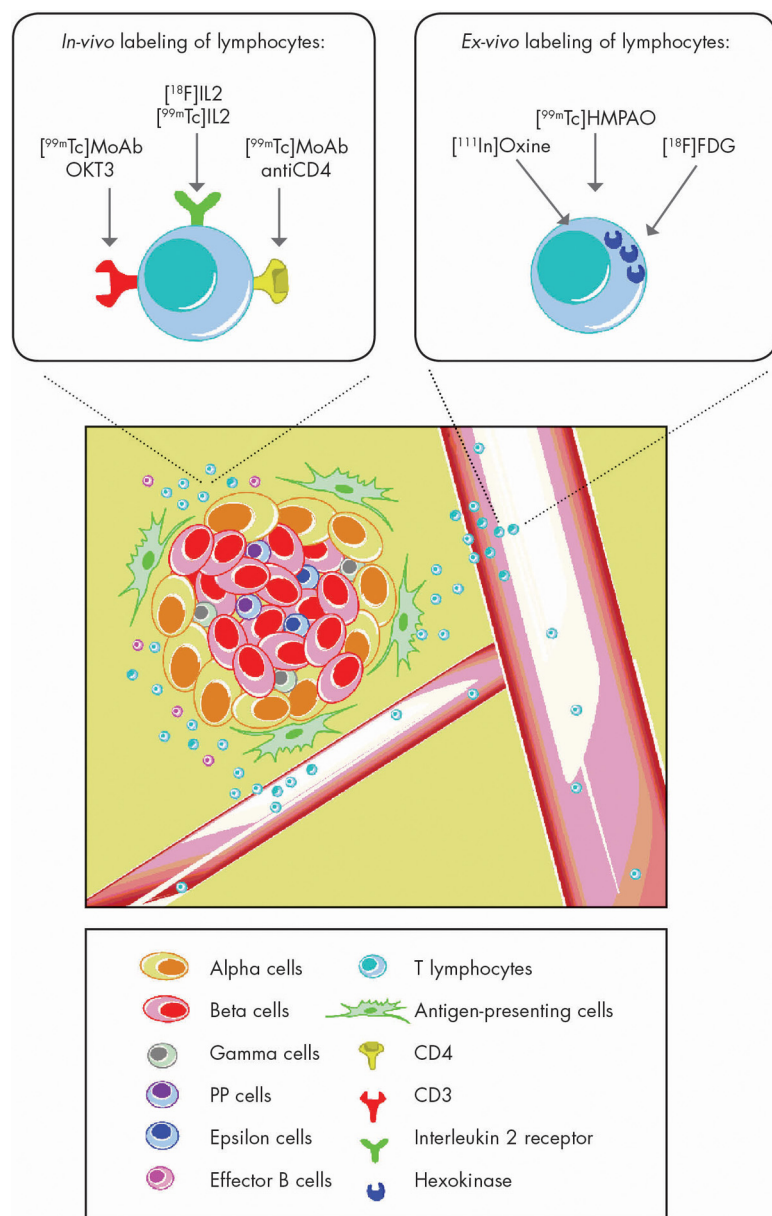
Figure 2.

Figure 2. Schematic structure of an islet of Langerhans infiltrated by B and T lymphocytes. The two drawings above show a T lymphocyte with radiopharmaceuticals used for targeting T lymphocytes *in vivo* (top left panel) and a T lymphocyte with radiopharmaceuticals used for labeling T lymphocytes *in vitro* (top right panel). In the first approach, the radiopharmaceutical is injected iv and binds to infiltrating cells in tissues. With the second approach, T cells are purified *in vitro* from peripheral blood, labeled, and then reinjected iv to migrate into the inflammatory site.

diopharmaceutical in the pancreas ($18.0 \pm 0.5\%$ ID/g), which could partially be blocked by administering an excess of the unlabeled compound. These results indicate that the uptake in the pancreas is receptor mediated, although it may involve another receptor than the sstr. The selectivity of this radiopharmaceutical was evaluated also in CHO cells that expressed either sstr1 or sstr2. The re-

sults showed that the affinity of the radioiodinated compound is higher for sstr1 than sstr2. When comparative biodistribution studies in NOD mice and control CBA/J mice were performed, a significantly lower uptake of the radiopharmaceutical was observed in 8- to 16-wk-old NOD mice compared with control mice. Moreover, in 26-wk-old NOD mice, hardly any uptake in the pancreas was detectable, which probably reflects the progressive decrease of BCM caused by the progression of diabetes. It should be noted that the uptake of this radiopharmaceutical was also high in organs that surround the pancreas, such as small intestine, liver, spleen, and kidney, hampering imaging quality of BCM (95).

2. Dopamine synthesis

L-3,4-Dihydroxyphenylalanine (L-DOPA) is a drug that is used for the treatment of Parkinson's disease and Parkinson's disease-related disorders (96–98). L-DOPA is efficiently converted into dopamine by the enzyme amino acid decarboxylase (AADC) (99). L-DOPA has been labeled with ^{11}C and ^{18}F for PET imaging of the integrity of dopaminergic system in the neurodegenerative disorders, because the L-DOPA uptake correlates with the density of dopaminergic neurons (100, 101). $[^{18}\text{F}]\text{DOPA}$ has also been successfully used for PET imaging of neuroendocrine tumors, like carcinoids, because these tumors have a high expression of AADC (102). AADC is also expressed in the granules of pancreatic β -cells, and therefore, this enzyme could be investigated as a target for β -cell imaging. Indeed, pancreatic accumulation of radiolabeled L-DOPA was investigated in mice. It was shown that tritiated L-DOPA accumulated selectively in pancreatic β -cells and other islet cells. Microscopic autoradiography showed that the radioactivity was predominantly localized in the secretory granules. Inhibition experiments proved that L-DOPA accumulation in the pancreas was AADC mediated (103–105).

Sweet *et al.* (73) have tested the *in vitro* uptake and retention of tritium-labeled L-DOPA and dopamine in pancreatic β -cells. In contrast to the aforementioned *in vivo* experiments, dopamine showed moderate selectivity for β -cells over control cells in these *in vitro* experiments, whereas L-DOPA did not show any selectivity at all. These contradictory *in vivo* and *in vitro* results

require further investigation. ^{18}F - and ^{11}C -labeled L-DOPA have not been investigated for β -cell imaging yet.

3. Serotonin

Serotonin receptors are predominantly expressed in the CNS, especially in the thalamus, hypothalamus striatum, and frontal cortex. Serotonin receptors are also found in tissues with neuroectodermic origin (endocrine cells) and tissues with extensive innervation, such as the gastrointestinal tract (106). The serotonin receptors in the pancreas are similar to those in the CNS, and all subtypes of serotonin receptor are expressed on the β -cell surface (106, 107).

Serotonergic neurons, γ -aminobutyric acid (GABAergic), glutamic-acid decarboxylase (GAD)-containing fibers are dense in periductal cells and periislet. In spontaneous animal models of T1D, *i.e.* NOD mice and BB/W rats, a loss of sympathetic nerves during the progression of diabetes was shown. For instance, there is a severe nerve loss in 10-wk-old NOD mice that show the first symptoms of diabetes (108).

Several PET radiopharmaceuticals have been developed for monitoring the distribution, density, and functional state of serotonin receptors in the CNS, including [^{11}C]WAY100635, 4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p- ^{18}F fluoro-benzamido]ethylpiperazine ([^{18}F]MPPF), and [^{18}F]altanserine, but none has been used for imaging BCM. Only [^3H]serotonin was used to investigate the feasibility of labeled serotonin analogs for β -cell imaging. *In vitro* autoradiography of the pancreas incubated with [^3H]serotonin showed radioactivity associated with β -cell secretory granules. Blocking studies with an AADC inhibitor reduced the number of grains that were visualized by autoradiography (104).

[^3H]Serotonin was also used in cell experiments with exocrine cells (PANC-1), endocrine cells (INS-1), and primary cell cultures from isolated rat islets. [^3H]Serotonin uptake was about twice as high in the endocrine cell line and cultures from freshly isolated islets than in the exocrine cells (73). Thus, imaging with labeled serotonin may be an interesting approach to monitor BCM, and indeed, recently, Deeney *et al.* (109) found a good correlation between insulin secretion and [^3H]serotonin uptake in INS-1 cells (endocrine β -cell line).

This observation could have interesting implications for imaging with [^{11}C]5-hydroxytryptophan ([^{11}C]HTP), the precursor of serotonin, which is successfully used for imaging neuroendocrine and islet-cell tumors (110, 111). [^{11}C]HTP is taken up by neuroendocrine cells via the large amino acid transporter and converted into serotonin by the enzyme AADC and then stored in secretory granules. Therefore, [^{11}C]5-HTP could be a good candidate for imaging the decrease of BCM during diabetes progression, although more investigations are needed.

D. Imaging vesicular monoamine transporters type 2 (VMAT)

VMAT are ATP-dependent transporters that regulate the uptake of monoamines from cytoplasm into secretory granules in neuroendocrine cells. This system is preferentially expressed in catecholergic, serotonergic, and histaminergic neurons as well as in endocrine cells (112). At the moment, there are only two known isoforms of this transporter, VMAT1 and VMAT2. The differential expression of VMAT1 and VMAT2 in neuroendocrine tissues was investigated by immunohistochemical analysis and *in situ* hybridization. In the pancreas, VMAT1 is expressed in duct cells, and it is not expressed in the pancreatic islets. In contrast, VMAT2 is preferentially expressed in β -cells but not in glucagon- and somatostatin-producing cells (113). In particular, it has been demonstrated that $88 \pm 4\%$ of β -cells are positive for VMAT2 ($29 \pm 15\%$ of total islet cells), but also $39 \pm 7\%$ of the exocrine duct cells are positive for VMAT2. These results demonstrated the correlation between pancreatic islet β -cells and VMAT2 expression, although the target does not appear to be totally specific for β -cells (114).

Dihydropyridylbenzazine (DTBZ) is a ligand that selectively binds to the VMAT2-binding site. Two radiopharmaceuticals were developed for PET imaging of VMAT2, [^{11}C]DTBZ, and 18F-9-fluoropropyl-(+)-dihydropyridylbenzazine, [^{18}F]FP-DTBZ. Historically, labeled DTBZ was used for PET imaging of the VMAT2 in the CNS of patients with neurodegenerative diseases, where a decreased uptake is observed due to the loss of monoamine neurons (115). Simpson *et al.* (116) have demonstrated that the [^{11}C]DTBZ is a promising PET radiopharmaceutical for β -cell imaging. [^{11}C]DTBZ was studied in Lewis rats treated with streptozotocin (STZ) as a model for diabetes and in normal euglycemic rats as a control group. STZ induces apoptosis in β -cells, resulting in an increase in glucose concentration in blood. If the glucose concentration is above 300 mg/ml, the rats are considered diabetic. PET imaging showed that the uptake of [^{11}C]DTBZ is decreased significantly ($P < 0.05$) in STZ-induced diabetic rats compared with the euglycemic control rats. VMAT2 and proinsulin mRNA were quantified by real-time quantitative RT-PCR. Animals treated with STZ showed lower relative abundance of VMAT2 and proinsulin transcripts and decreased functionality and viability of β -cells. These data suggest that [^{11}C]DTBZ uptake is correlated with the presence of VMAT2 expression on β -cells. Longitudinal noninvasive PET studies in BB/W diabetes-prone rats with [^{11}C]DTBZ were performed by Souza and co-workers (117) in an attempt to image BCM decline with time. The studies were started in 4-wk-old animals, when they were still euglycemic, and continued until the animals were diabetic. Results showed a good correlation between the pancreatic radiopharmaceutical uptake of [^{11}C]DTBZ and VMAT2 expression ($r^2 = 0.58$), which declined during disease progression. Interestingly, a sig-

nificant reduction of pancreatic uptake was already detectable before the appearance of hyperglycemia, thus suggesting the use of this radiopharmaceutical to predict diabetes before its clinical onset.

Cross-sectional human studies were also performed in nine control healthy volunteers and six patients with long-lasting T1D. The pancreatic binding potential of [^{11}C]DTBZ in patients was only 14% lower than in healthy controls. Because BCM in patients with advanced disease is minimal, a larger reduction in the diabetic patients' pancreatic binding potential was expected. The small reduction in radiopharmaceutical uptake could be explained by a nonspecific radiopharmaceutical uptake in the exocrine portion of the pancreas and by the presence of a radioactive [^{11}C]DTBZ catabolite. Despite the fact that animal studies are partially in disagreement with studies in humans, more studies are needed to clarify the overestimation of pancreatic radiopharmaceutical uptake in patients (117–119). Taken together, these results suggest that DTBZ may not be sensitive enough to monitor changes of BCM in the early stages of the disease.

Because of the short half-life of ^{11}C , a fluorinated analog of DTBZ was also developed, which can be distributed to nuclear medicine centers that do not have a cyclotron for ^{11}C production. Like [^{11}C]DTBZ, [^{18}F]FP-DTBZ was primarily used for studying VMAT2-positive neurons in the brain.

Recently, a first preliminary study on imaging of β -cells with [^{18}F]FP-DTBZ was published. Kung *et al.* (115) have demonstrated in rats that [^{18}F]FP-DTBZ has potential as a radiopharmaceutical for β -cell imaging. Biodistribution and small-animal PET imaging studies in normal rats showed that the uptake of [^{18}F]FP-DTBZ in the pancreas was maximum at 30 min after injection ($5.50 \pm 0.97\%$ ID/g). The data also showed significant uptake in the organs around the pancreas, such as liver, spleen, and kidney (2.82 ± 0.24 , 1.03 ± 0.18 , and $1.06 \pm 0.06\%$ ID/g, respectively), but uptake in these organs was lower than in pancreas. Blocking studies with FP-(+)-DTBZ and (+)-DTBZ were performed to confirm specific binding of fluorinated DTBZ to VMAT2. There was a significant reduction in uptake of [^{18}F]FP-DTBZ after the treatment with both unlabeled compounds, demonstrating that the binding of [^{18}F]FP-DTBZ is specific. Pretreatment with the inactive enantiomer did not result in a significant reduction of radiopharmaceutical uptake, which means that radiopharmaceutical uptake is stereo-selective (115). Finally, a preliminary study in human pancreatic islet homogenates demonstrated high-affinity binding of [^{18}F]FP-DTBZ to its specific target. Finally, Eriksson *et al.* (120) have investigated the feasibility of using [^{18}F]FE-(+)-DTBZ as a probe for BCM in human T1D and T2D tissue

slices. They demonstrated that the binding of the radiopharmaceutical to its target is not specific, with high accumulation in the exocrine pancreas. Animal studies showed significant defluorination as indicated by high bone uptake. Thus, [^{18}F]FE-(+)-DTBZ does not appear to be a suitable radiopharmaceutical for the detection and quantification of BCM.

E. Imaging presynaptic vesicular acetylcholine transporters

The maintenance of glucose homeostasis is regulated by an interplay between different factors, such as the glucose concentration, autonomic nervous system, systemic growth factors, and autocrine and paracrine actions of molecules produced by β -cells.

The peripheral autonomic nervous system regulates many physiological functions including the endocrine apparatus. Within the pancreas, the cholinergic system is found predominately in the periphery of the islets, where it regulates β -cell function. Several studies demonstrated the presence of choline acetyltransferase and acetylcholine esterase activity in islet cells, suggesting an important role of the cholinergic system in the regulation of insulin secretion. Acetylcholine plays a central role in insulin secretion in the absorptive phase via M_3 muscarinic receptors on pancreatic β -cells (121, 122).

N-[^{18}F]Fluorobenzylatrozamicol is a radiopharmaceutical that binds specifically to the vesamicol receptor associated with the vesicular acetylcholine transporters. Preliminary data showed an excellent pancreatic uptake between 10 and 90 min after [^{18}F]fluorobenzylatrozamicol injection, but there was also a significant salivary gland, liver, and gallbladder uptake. In T1D, cholinergic innervation is reduced, and it will be interesting to further study this radiopharmaceutical in this disease (123).

F. Imaging β -cell metabolic pathways

1. Fluorodeoxyglucose (FDG)

The introduction of [^{18}F]FDG for PET imaging has revolutionized the clinical diagnosis of several pathologies. The most important use of this radiopharmaceutical is in diagnosis, staging, and therapy evaluation in cancer (124, 125). Nevertheless, Sweet *et al.* (73) used FDG labeled with tritium for preliminary *in vitro* studies to image β -cells, but the compound was not selective for β -cells, because there was high accumulation and retention also in exocrine cells. In 2005, the feasibility of [^{18}F]FDG as a probe to image the islets of Langerhans was explored. The aim of this study was to follow the cell trafficking and the fate of islets after intraportal transplantation. Results showed that radioactivity in the liver was higher after islet transplantation than in controls. A major problem was the

radiopharmaceutical washout, because 50% of activity was released from the islets every hour after the injection (126). In 2007, Eich *et al.* (127) applied hybrid PET/CT to visualize transplanted porcine islets labeled with [^{18}F]FDG after intraportal infusion. Only 50% of the infused radioactivity was found in the liver 20 min after injection, indicating that significant loss of islets already happens soon after transplantation. The immediate action of the innate immune system (complement system) on the transplanted islets, or hypoxia-induced cell death, could explain the huge loss of islets after transplantation. Moreover, a heterogeneous distribution of the radioactivity in the liver was observed, defined as a hot spot, which represents groups of islets trapped in the portal branches of the liver.

In 2009, the same group confirmed that transplanted islets are not distributed uniformly in the liver, but are distributed differently between the segments and lobes of the patient's liver. Moreover, they were able to quantify the distribution pattern of transplanted islets by PET/CT (128). In conclusion, a dynamic PET/CT investigation could be useful for quantitative and qualitative analysis of islet survival after transplantation, but [^{18}F]FDG is not a suitable candidate for imaging the residual BCM in TD1 patients.

2. Nicotinamide

Nicotinamide is a molecule that belongs to the B3 vitamins family. Nicotinamide protects the cell from reactive oxygen species and nitric oxide and probably improves cell regeneration (129).

In mice treated with STZ, nicotinamide prevents the reduction of nicotinamide adenine dinucleotide in pancreatic islet cells.

Autoradiographic studies, using [^3H]2-deoxyglucose and [^{14}C]nicotinamide showed that these compounds compete for the same transporter (glucose transporter 1). Nicotinamide has the same trans-membrane transport mechanism as 2-deoxyglucose (130). Nevertheless, Sweet *et al.* (73) have demonstrated *in vitro* that the uptake of [^{14}C]nicotinamide in islet pancreatic cell lines and exocrine pancreatic cell lines is not significantly different. Thus, labeled nicotinamide does not appear to be a suitable tool for β -cell imaging.

G. Imaging GLP-1 receptor (GLP-1R)

GLP-1R is a G protein-coupled receptor that binds the incretin hormones released from the intestinal tract during digestion. These receptors are important in the regulation of glucose homeostasis, through insulin, glucagon, and somatostatin secretion. GLP-1R triggers an important intracellular signaling pathway for the β -cell proliferation and differentiation (131, 132). Binding studies showed

that this receptor is widely distributed in several organs. It is located in brain, hypothalamus, stomach, heart, intestine, kidney, and pancreatic islets (133). Colocalization studies of insulin, glucagon, somatostatin, and pancreatic polypeptide with GLP-1R mRNA and protein demonstrated that GLP-1R expression is restricted to the β -cells, whereas the level of receptor expression on α - and δ -cells is below the detection limit. Weak and heterogeneous immunoreactivity was also observed in the pancreatic duct membrane (134). There are several ligands that can bind the receptor: GLP-1, GLP-1 mimetics, exendin-4, and several new compounds with higher biological half-life and higher resistance to the inactivation by proteases (131). These pharmaceuticals are used for GLP-1-based therapy, and at present, GLP-1 analogs have attracted the attention of nuclear medicine physicians for their possible role as β -cell imaging probes.

To this purpose, GLP-1 was labeled with ^{125}I . This radiopharmaceutical was tested in an insulinoma cell line (RINm5F) *in vitro* and *in vivo* (NEDH rats inoculated with RINm5F cells). Both experiments showed high and specific uptake, but a serious problem was the high lysosomal degradation rate of the radiopharmaceuticals (135).

At present, studies are in progress to increase the stability of these labeled proteins. For example, dipeptidyl peptidase IV can be inhibited to prevent the cleavage of GLP-1 in body fluids and thus to increase its biological half-life. Recently Brom *et al.* (136) reported the development of a new radiopharmaceutical, Lys 40 -(DOTA)exendin-3 labeled with ^{68}Ga . There was a specific and high uptake in a sc INS-1 insulinoma, and high uptake in the kidney was found, because of the renal clearance of the radiopharmaceutical. This new radiopharmaceutical might be also used to image BCM in T1D, but this has not been investigated yet. For clinical applications, however, a stable peptide with better biological properties may be required. For this purpose, Wild *et al.* (137) investigated a GLP-1 analog designed to be more stable in human serum than other GLP-1 ligands. Lys 40 -amino hexanoic-diethylenetriaminepentaacetic acid (Ahx-DTPA)-NH $_2$ -exendin-4 was labeled with ^{111}In and tested in Rip1Tag2 transgenic mice. This labeled peptide proved to be more stable than radiolabeled GLP-1 and exendin-3. More than 80% of radiolabeled exendin-4 remained intact in human serum after 4.5 h. The biodistribution of this radiopharmaceutical showed high uptake in insulinoma, the target tissue for this radiopharmaceutical. And uptake was reduced more than 95% when the animals were treated with an excess of the unlabeled ligand. High radiopharmaceutical uptake was also observed in the kidney, but this uptake was not specific, because it was not blocked by the unlabeled ligand. The tumor-to-pancreas uptake ratio for this

radiopharmaceutical was extremely high, 13.6 at 4 h after radiopharmaceutical injection. In 2010, Pattou *et al.* (138) published a case report describing the successful clinical application of radiolabeled exendin-4 for imaging autologous islets transplanted in the brachioradialis muscle. One year after transplantation, focal accumulation of [Lys⁴⁰-(Ahx-DTPA¹¹¹In)-NH₂]exendin-4 was still visible in the transplantation site. These results provide clinical evidence that GLP-1 analogs could be useful for islet transplantation imaging. Whether pancreatic uptake of the exendin-4 analog is sufficient for BCM imaging remains to be investigated. Although pancreatic β -cells express high levels of GLP-1R, it might be difficult to visualize them with this radiopharmaceutical because of the limited resolution of SPECT and the small size of the pancreatic islets. We can conclude that the GLP-1 analogs are promising peptides for β -cell imaging due their high specificity for the β -cells, but a PET radiopharmaceutical may be required. To this purpose, exendin-3 was labeled with ⁶⁸Ga by Brom *et al.* (136), as described above. Because ⁶⁸Ga is only a partial positron emitter, producing high-energy positrons, perhaps even better image quality could be achieved when a pure positron emitter such as ¹⁸F or ¹¹C is used for labeling. Gao *et al.* (139) recently published the first ¹⁸F-labeled PET radiopharmaceutical for GLP-1. These authors labeled EM3106B, a GLP-1 analog, with ¹⁸F through conjugation with N-2-(4-[¹⁸F]fluorobenzamido)ethylmaleimide. The ¹⁸F-labeled EM3106B showed specific binding to the GLP-1R on insulinoma cells *in vitro* and *in vivo*. Specific uptake in insulinoma in tumor-bearing mice could be clearly delineated by PET. So far, however, no reports on imaging of β -cells with this PET radiopharmaceutical have been published. Despite promising results so far, an intrinsic complication of this class of radiopharmaceuticals for β -cell imaging could be the presence of the GLP-1R on the pancreatic duct cells in the exocrine pancreas, which might obscure the signal originating from β -cells, especially when BCM is reduced.

H. Imaging β -cells with islet-cell-specific antibodies

Antibodies are heavy (~150 kDa) globular plasma proteins known as Ig. They can recognize specific epitopes through their variable regions. To quantify BCM, radiolabeled antibodies need to bind to specific target molecules selectively expressed on the β -cell surface. Several β -cell-specific biomarkers are known, and specific antibodies against these epitopes have been developed.

An interesting antibody is the IgM κ -monoclonal antibody IC2 against sulfatide expressed on the β -cell surface. Sulfatide is a glycosphingolipid localized in secretory granules and on the plasma membrane of β -cells. Sulfatide controls the secretion of insulin (140). No immunoreac-

tivity was found in frozen pancreas sections, because this biomarker is highly sensitive to detergent, denaturant, and other chemical reagents used during the staining treatment. Several *in vitro* and *in vivo* studies with this antibody were done. IC2 was labeled with ¹²⁵I for pharmacokinetic studies and with ¹¹¹In for *in vivo* and *in vitro* studies.

Experiments with these probes showed excellent uptake and specificity of the radiopharmaceutical, but the most interesting result was the high correlation between accumulation of the labeled antibody in the pancreas and the BCM in diabetic animals ($r^2 = 0.936$) (141, 142). This antibody is not commercially available, and only animal studies were performed so far due to the xenogeneic origin of this IgM antibody. If this antibody was injected in humans, it would probably develop an immune reaction due to its rat origin. The promising results obtained so far warrant adaptation of the IC2 antibody for human use. Another limitation of radiolabeled pancreatic antibodies for imaging β -cells is high background activity due to the slow clearance of antibodies from circulation. This could be done using only the variable region (Fv) of the antibody or a humanized version of it.

So far, no other radiolabeled antibodies for imaging of β -cells have been reported.

I. Imaging β -cells using radiolabeled peptides

Because of this slow plasma clearance, monoclonal antibodies have to be labeled with long-lived isotopes, resulting in a high radiation burden to the patient. To overcome these limitations, pretargeting strategies could be applied. First, pancreatic β -cells are targeted by a specific antibody, followed by the administration of a small labeled effector that specifically binds to the antibody previously injected (142). Liu *et al.* (143) report they used CC49 as an antibody against tumor-associated glycoprotein 72, a glycoprotein expressed in a variety of adenocarcinoma and also present on the surface of β -cells. They modified the antibody by conjugation with a morpholino oligomer (MORF). The effector is a complementary MORF (cMORF) labeled with ^{99m}Tc or ¹¹¹In. The results of this approach were promising, with a high accumulation of labeled cMORF in the pancreas. The main problem of [^{99m}Tc]cMORF was the high accumulation of this labeled effector in the intestines (2% of the total injected dose). Thus, the quantification of the BCM was hampered by the high background activity around the pancreas. In contrast, ¹¹¹In-labeled cMORF did not show any intestinal accumulation (144). Therefore, the application of pretargeting strategies for BCM quantification could be feasible using ¹¹¹In-labeled cMORF, but further investigation is needed to validate this new and promising methodology.

J. Concluding remarks on nuclear medicine techniques for β -cell imaging

The design of a new radiopharmaceutical for imaging β -cells follows the histological identification of a specific β -cell antigen or a specific metabolic pathway. There are also surrogates of BCM that can be targeted, such as neurotransmitter receptors. Nuclear medicine techniques, both SPECT and PET, do not have the special resolution necessary for islet imaging, and therefore, nuclear medicine probes may have a role in islet imaging only if they provide a very high target/background ratio. The smaller the anatomical structure to image, the higher must be the target/background ratio (ratio between the radiopharmaceutical uptake in the endocrine and exocrine tissue). In this context, the most promising radiopharmaceuticals tested so far are serotonin receptor agonists and GLP-1 analogs, although competing with MRI technology is a big challenge.

V. Nuclear Medicine Techniques for Imaging Insulinitis

Insulinitis is the lymphomononuclear cell infiltration of the pancreatic islets, causing β -cell destruction. Insulinitis can begin a long time before the clinical manifestations of diabetes and is the pathological hallmark of T1D (Table 1). It is an autoimmune process regulated by many factors, such as a genetic predisposition and environmental factors (145). The current status of knowledge on T1D histopathology is mostly based on old observations from the 1960s (146–149). The major problem for studying the natural history of insulinitis in humans is the limited availability of the pancreatic specimens from diabetic patients and prediabetic subjects because of the difficulty in obtaining pancreatic biopsies and in identifying subjects at risk to develop diabetes. Mostly postmortem pancreatic material from diabetic patients has been analyzed to study in detail the characteristics of insulinitis (150). Histological examination showed the presence of insulitis in more than 50% of the T1D patients at the time of diagnosis, indicating that the insulitis is a crucial aspect in the pathogenesis of diabetes. Because in the last decades many biological therapies have been identified to modulate the immune process in several autoimmune diseases, these could also be applied in T1D if patients could be identified before the complete destruction of BCM. Early diagnosis of insulitis is, therefore, mandatory. New strategies are needed to detect it, not only for prevention purposes but also for therapy decision making and therapy follow-up.

From previous sections, we learned of the frustrating attempts of radiological and optical techniques for imag-

ing lymphocyte trafficking *in vivo* in humans. This is mainly due to the difficulty of coupling enough contrast agent (or fluorescent dye) to lymphomononuclear cells to produce a signal detectable from outside the body. Nuclear medicine, by using radiolabeled probes (with high specific activity) that target specific cell antigens, is the only concrete possibility to image insulitis in humans.

Probes for noninvasive imaging include labeled autologous lymphocytes, labeled cytokines, and labeled antibodies and peptides against lymphocyte antigens and FDG, as summarized in Table 3 and Fig. 2 and described in detail below.

A. Labeled lymphocytes

Autoreactive T cells are implicated in the inductions of β -cell apoptosis during the prediabetic phase (151). A direct approach to image insulitis can be the use of *ex vivo* radiolabeled autologous lymphocytes. This implies the purification of $40\text{--}100 \times 10^6$ autologous lymphocytes from peripheral blood, their *in vitro* labeling of [^{111}In]oxine (152–154), [^{111}In]tropolonate (155, 156), or [$^{99\text{m}}\text{Tc}$]HMPAO (hexamethylpropyleneamine oxime) (157–159) and their iv readministration into the patient. The assumption is that peripheral blood lymphocytes are able to migrate to the affected tissue attracted by various cytokines and chemokines and home there (160). Indeed, lymphocytes from patients affected by T1D were labeled with [^{111}In]oxine and reinjected in the same patient. After the autologous transfer, the homing of labeled lymphocytes into the pancreas was, however, poor. Only a few patients were examined, and these data were never confirmed again by others (161). Others studied the homing of labeled lymphocytes in BB/W rats by investigating the pancreatic localization of autologous labeled lymphocyte after reinfusion in diabetes-prone, resistant BB/W rats and in Wistar Furth rats. All groups of animals showed similar lymphocyte uptake in the pancreas. There was no correlation between the insulitis and the amount of lymphocytes localized in the pancreas (162). The interpretation of these results was that only a few circulating cells are able to migrate into affected tissues during an autoimmune disease, whereas most cells migrate to the spleen and other secondary lymphoid tissues. This was also investigated in Hashimoto's thyroiditis and Graves's disease (160). An alternative hypothesis is that *ex vivo* labeled lymphocytes with [^{111}In]oxine are damaged by the radiations and the purification procedure and lose their migration capacity when reinjected *in vivo*. Therefore, this technique was abandoned but provided a basis for nuclear medicine imaging of insulitis.

TABLE 3. Radiopharmaceuticals used for insulinitis imaging

Radiopharmaceutical	Isotope	Type of study	Target	Pros	Cons	Ref.
Autologous lymphocytes	^{111}In	Animal studies in BB/W	PBL	Feasibility study	Low uptake	162
Autologous lymphocytes	^{111}In	T1D at onset	PBL	PBL can home in the pancreas	Toxicity on PBL, low uptake, few patients	161
IL-2	^{123}I	<i>In vitro</i> and NOD and BB/W rats pre-T1D	IL2R or CD25	Good binding <i>in vitro</i> to TAC, ARG, good <i>ex vivo</i> data, good comparison with αLA , kinetics, and ARG; good concordance with metabolic markers	Not ideal imaging tool, very expensive; pancreas ROI identified by CT or MRI	166–170
IL-2	$^{99\text{m}}\text{Tc}$	T1D, LADA, non-T1D, pancreas cancer	IL2R or CD25	Good correlation with clinical and metabolic data, correlation with histology	High liver uptake, few LADA patients	172, 173
IL-2	^{18}F	Animal studies, BB/W rats and NOD mice	IL2R or CD25	Linear correlation with the diabetes progression, correlation with the extent of insulinitis	Preliminary studies	200
Human Ig	$^{99\text{m}}\text{Tc}$	T1D and pre-T1D	Fc receptor	Good uptake and results comparable to IL-2	Long distribution time	176, 177
FDG	^{18}F	Animal studies	GLUT 1	Good pancreatic uptake	No correlation with the degree of insulinitis	182, 183
FDG	^{18}F	Newly diagnosed type 1 diabetes	GLUT 1	Good uptake correlated and correlation with the regression of insulinitis and radiopharmaceutical uptake	Weak methodology, poor pancreatic localization	184

GLUT1, Glucose transporter 1; IL-2R, IL-2 receptor; PBL, peripheral blood lymphocytes; ROI, regions of interest; TAC, T-activated cells; ARG, autoradiography; αLA , α -lactalbumin; LADA, latent autoimmune diabetes.

B. Interleukin-2

IL-2 is glycoprotein of 133 amino acids mainly produced by activated T lymphocytes. It plays a central role in T-cell growth, survival, differentiation, and transcriptional regulation. The IL-2 receptor consists of three subunits (α -, β -, and γ -chain; respectively, CD25, CD122, and CD132) with different binding affinities. The highest affinity binding of IL-2 is with CD25 that can be present as a trans-membrane or soluble receptor. CD122 is implicated in the intracellular signal transduction, and CD132 is a common chain-cytokine receptor (163, 164). The intracellular pathway of the activation-transduction of T cells needs the entire heterotrimeric complex formed by all of the subunits. The complex triggers an intracellular signal through the activation of JAK/STAT, PI3K, and MAPK pathways. These pathways lead to cell proliferation and increase the lymphokine secretion and MHC class-II molecule expression. All of these properties are important to regulate the immune response in autoimmune conditions (165).

Radiolabeled IL-2 was used for imaging of several chronic inflammatory conditions, characterized by the

lymphomononuclear cell infiltration of CD25-positive cells including T1D (166).

First, IL-2 was labeled with ^{123}I . BB/W rats and NOD mice were used as models of diabetes. BB/W rats have characteristics that are closer to human insulinitis, although they show a lower degree of insulinitis than NOD mice. In NOD mice, radiopharmaceutical uptake could be quantified well due to the high lymphocytic infiltration. [^{123}I]IL-2 showed good *in vitro*, *in vivo*, and *ex vivo* data, including high specific binding, good retention, and good correlation between the radiopharmaceutical uptake in the pancreas and the severity of insulinitis (167–170). This radiopharmaceutical was tested in pre-T1D patients and prediabetic subjects with high risk to develop this pathology (islet serum markers were checked). [^{123}I]IL-2 pancreatic uptake was clearly distinguished from the kidney and spleen (17, 166).

Chianelli *et al.* (171) radiolabeled IL-2 with $^{99\text{m}}\text{Tc}$. The first step of the labeling procedure is the conjugation of IL-2 with N3S, and the second step is the labeling of conjugated product with $^{99\text{m}}\text{Tc}$. The radiopharmaceutical

had a high specific activity (64 GBq/ μ mol) and good receptor-binding properties. [^{99m}Tc]N3S-IL-2 was evaluated in newly diagnosed T1D patients before and 1 yr after treatment with nicotinamide and insulin therapy. The results of these studies showed good pancreatic radiopharmaceutical uptake due to the lymphomononuclear infiltration at baseline and a progressive uptake reduction during the immunomodulatory treatment (172, 173). An improvement of the labeling procedure of [^{99m}Tc]IL-2 was achieved using the bifunctional chelating agent 6-hydrazinopyridine-3-carboxylic acid, resulting in a simplified radiolabeling procedure and an easily available stable compound (174). [^{99m}Tc]6-Hydrazinopyridine-3-carboxylic acid-IL-2 has not been evaluated as an insulinitis imaging agent yet. Radiolabeled IL-2 could be a promising radiopharmaceutical to detect infiltrating lymphocytes in insulinitis. A major problem remains the low resolution and sensitivity SPECT and the small size of the pancreatic islets.

For that reason, recently, Di Galleonardo *et al.* (175) have synthesized a new radiopharmaceutical [^{18}F]FB-IL-2 [N-(4- ^{18}F -fluorobenzoyl)interleukin-2 by conjugation of N-succinimidyl 4- ^{18}F fluorobenzoate to lysine residues in the IL-2 protein. Good stability in human plasma and retention of the biological activity was observed. Preliminary animal studies show good pancreatic uptake, but additional animal studies are needed for a better evaluation of this radiopharmaceutical.

C. Labeled antibodies

Antibodies can be divided in two groups: polyclonal and monoclonal antibodies. Polyclonal antibodies are nonspecific Ig that recognize the Fc receptor in the infiltrating lymphocytes.

Human polyclonal Ig radiolabeled with ^{99m}Tc was used successfully in many autoimmune diseases (176). Barone *et al.* (177) proved that this radiopharmaceutical can be used to detect the lymphomononuclear cell infiltration in T1D patients. Moreover, this study demonstrates the feasibility of selection of patients that can benefit from immunomodulatory therapy.

Malviya *et al.* (178) have recently published a non-Fc-binding anti-CD3 monoclonal antibody labeled with ^{99m}Tc that was suitable for imaging of small numbers of T lymphocytes in tissues. Likewise, the monoclonal antibodies OKT3 and anti-CD4 were also radiolabeled with ^{99m}Tc and ^{111}In to target these specific receptors on the T lymphocyte surface (178–180). These antibodies were used for imaging autoimmune diseases that are associated with strong inflammatory reactions, although insulinitis was not investigated. For insulinitis imaging, the main problem is the relatively low number of lymphocytes or the relatively low

number of antigens expressed on lymphocytes present in the insulinitis that can be detected with these radiopharmaceuticals. Indeed, the resolution of nuclear medicine techniques stands mainly on the signal-to-noise ratio, and radiolabeled antibodies are known for having a high background, circulating activity. Also, the poor resolution of SPECT could be another limiting factor, and the higher resolution of PET could be advantageous. In this regard, differently from cytokines, antibodies have a long biological half-life (from 5–20 d) and consequently a long distribution time; therefore, PET isotopes, with short half-life (^{11}C or ^{18}F), are not suitable for labeling antibodies, because the physical half-life of the isotope is much shorter than the biological half-life of the antibody. Long-lived isotopes, like ^{89}Zn , could be useful but give rise to high radiation exposure.

Despite promising preliminary data, labeled antibodies still need to be validated as suitable candidates for imaging of insulinitis.

D. [^{18}F]Fluorodeoxyglucose

Different inflammatory cell types exhibit increased glucose metabolism in sites of inflammation during several pathologies (181). The glucose pathway might be used as a potential target to detect subacute and chronic inflammation.

Kalliokoski *et al.* (182) investigated the feasibility of [^{18}F]FDG as a radiopharmaceutical for the detection of infiltrating lymphocytes during insulinitis in a diabetes-prone animal model (NOD mice). They found a 2.3-fold increase in radiopharmaceutical uptake in the endocrine pancreas compared with the exocrine tissue. Similar uptake was found in early-stage and late-stage insulinitis, and unfortunately, the islet-to-exocrine pancreas uptake ratio did not correlate with the degree of the insulinitis ($r = 0.0068$). In addition, animal studies in rats proved that this radiopharmaceutical cannot be used as a probe to detect the insulinitis, probably due to the low lymphomononuclear cell infiltration in the rat pancreas (182, 183). Still, Kalliokoski *et al.* (184) continued the investigation in humans. Sixteen newly diagnosed T1D normoglycemic patients were chosen as a test group (assuming they still had insulinitis at time of diagnosis), and nine age-matched healthy subjects were chosen as control group. The [^{18}F]FDG uptake in the pancreas of newly diagnosed diabetic patients was higher than controls and decreased after 3–6 months of insulin treatment.

From these studies, we can conclude that [^{18}F]FDG could be used for imaging of insulinitis, although combined analysis with techniques that allow anatomical localization of the pancreas may be required. Because inflammatory cells can be labeled efficiently with [^{18}F]FDG, Toso

and co-workers (185) performed a study to assess whether leukocytes *ex vivo* labeled with [^{18}F]FDG were able to detect graft rejection after islet transplantation. This study clearly demonstrated that leukocytes (mainly granulocytes) labeled with [^{18}F]FDG could not detect inflammation after transplantation, most likely due to the presence of lymphomononuclear cells in the inflammatory reaction. This technology therefore appears not sensitive and specific enough to allow the visualization or the quantification of the inflammatory response after islet transplantation.

E. Concluding remarks on nuclear medicine techniques for insulinitis imaging

Similarly to the β -cell imaging approach, the imaging of islet-infiltrating cells requires the preliminary knowledge of leukocyte antigens and of histopathology of the disease during its natural history. Most of these data are available from animal models and are extrapolated to humans with some limitation. The relevant cells infiltrating the islets are macrophages, cytotoxic T lymphocytes, and activated T lymphocytes. There are many different antigens expressed on these cells, and only a few radiopharmaceuticals have been designed to specifically target a few cells and antigens. Results are encouraging, particularly using radiolabeled IL-2, but this field of research needs to be further explored in humans.

VI. General Conclusions

Type 1, or juvenile (insulin-dependent), diabetes is a metabolic disease associated with the autoimmune destruction of β -cells. Because of the increase in the incidence of this pathology, it becomes necessary to develop new diagnostic tools to quantify the residual BCM and the chronic lymphocytic infiltration during the disease progression and in its preclinical phase. Until now, serological evaluation of autoantibodies (glutamic-acid decarboxylase auto-antibodies, insulin auto-antibodies, islet antigen-2 auto-antibodies) and indirect metabolic studies on the functionality of β -cells are performed after the clinical onset of the disease when the reduction of functional BCM might already be advanced to 60–80%. Early diagnosis of diabetes is necessary to design new therapeutic strategies for decreasing the inflammatory reaction against the β -cells and to evaluate the residual BCM. The aim is to block the activation of the immune system to keep the BCM viable and prevent disease progression. The final goal will be to develop a specific therapy that can improve the quality of life of patients or even obtain diabetes prevention.

For imaging BCM, the ideal method should discriminate between the endocrine and exocrine tissue (have a high target-to-background ratio) and between functional and nonfunctional β -cells. In this review, we surveyed the radiological, optical imaging, and nuclear medicine diagnosis methodology described until now. The major challenge is to find a technique that can combine specific targeting for β -cells with the high spatial resolution needed to localize the β -cells in islets of Langerhans spread throughout the pancreas parenchyma. With respect to the spatial resolution, MRI is the most promising technique, being noninvasive and with the highest spatial resolution and good soft tissue contrast. However, this technique lacks the specificity of the probes used to target β -cells. By contrast, PET and SPECT techniques can exploit radiopharmaceuticals with high specificity for β -cells but lack of resolution. Unfortunately, the ideal method/probe for imaging BCM is not available yet.

This review also highlights the available methods for insulinitis imaging. In this field, very little has been published with radiological techniques, and all hopes reside in nuclear medicine techniques by the use of specific PET and SPECT radiopharmaceuticals that bind *in vivo* to infiltrating immune cells in the insulinitis. Because insulinitis is characterized by the infiltration of activated T lymphocytes and macrophages in the pancreas, several radiopharmaceuticals that target inflammatory markers have been investigated. Because insulinitis is characterized by the infiltration of activated T lymphocytes and macrophages in the pancreas, several radiopharmaceuticals that target inflammatory markers have been investigated. The possibility to image the insulinitis (*i.e.*, the immune cells infiltrating the endocrine pancreas) may have an important role in identifying subjects at risk to develop T1D in which to start an immune therapy to delay or prevent the clinical onset of the disease. The therapeutic modulation of insulinitis requires surrogate markers to monitor the early efficacy of these therapies. In this view, insulinitis imaging could be one of the most relevant of these surrogate markers. These techniques could also help with therapy decision making, selecting the best drug depending on the kind of immune cells infiltrating the islets, and could help to follow up the efficacy of preventive therapies. These techniques could also help for therapy decision making, selecting the best drug depending on the kind of immune cells infiltrating the islets, and could help to follow up the efficacy of preventive therapies.

Finally, methods that can allow us to detect insulinitis or BCM could also be applied to monitor graft rejection after the islet or pancreas transplantation, which is the most promising therapeutic procedure under investigation for the replacement of β -cell function.

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