Generation of Novel Single-Chain Antibodies by Phage-Display Technology to Direct Imaging Agents Highly Selective to Pancreatic β- or α-Cells In Vivo

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OBJECTIVE—Noninvasive determination of pancreatic β-cell mass in vivo has been hampered by the lack of suitable β-cell–specific imaging agents. This report outlines an approach for the development of novel ligands homing selectively to islet cells in vivo.

RESEARCH DESIGN AND METHODS—To generate agents specifically binding to pancreatic islets, a phage library was screened for single-chain antibodies (SCAs) on rat islets using two different approaches. 1) The library was injected into rats in vivo, and islets were isolated after a circulation time of 5 min. 2) Pancreatic islets were directly isolated, and the library was panned in the islets in vitro. Subsequently, the identified SCAs were extensively characterized in vitro and in vivo.

RESULTS—We report the generation of SCAs that bind highly selective to either β- or α-cells. These SCAs are internalized by target cells, disappear rapidly from the vasculature, and exert no toxicity in vivo. Specific binding to β- or α-cells was detected in cell lines in vitro, in rats in vivo, and in human tissue in situ. Electron microscopy demonstrated binding of SCAs to the endoplasmatic reticulum and the secretory granules. Finally, in a biodistribution study the labeling intensity derived from [125I]-labeled SCAs after intravenous administration in rats strongly predicted the β-cell mass and was inversely related to the glucose excursions during an intraperitoneal glucose tolerance test.

CONCLUSIONS—Our data provide strong evidence that the presented SCAs are highly specific for pancreatic β-cells and enable imaging and quantification in vivo. Diabetes 58:2324–2334, 2009

The ability to noninvasively measure pancreatic β-cell mass in vivo may provide an early diagnostic tool for the diagnosis of type 1 diabetes and potentially promote the development and evaluation of novel therapeutic strategies. However, despite enormous efforts by various groups, none of the approaches tested have permitted a reliable and noninvasive assessment of β-cell mass in humans.

The small size of islets (50–300 μm in diameter), their low abundance (1–2% of pancreatic mass), and their scattered distribution throughout the pancreas create technical challenges for noninvasive imaging of β-cell mass. Because pancreatic islets possess no intrinsic contrast from the surrounding exocrine tissue, imaging techniques have focused on detecting the pancreatic islet with exogenous agents that are preferentially bound or concentrated in islets, and an extensive library of radiolabeled agents has been applied to islet imaging. However, radiolabeled compounds targeting the sulfonylurea receptor (1–5), the presynaptic vesicular acetylcholine transporter (6), and dopamine uptake in synaptic vesicles (7) have not proven to be effective for islet imaging, most likely because of their failure to achieve the required endocrine-to-exocrine binding ratio (>100:1), as suggested by Sweet et al. (8,9). Another proposed strategy to image islets within the pancreas relates to radiolabeled peptides derived from glucagon-like peptide 1 (GLP-1) or its analogues, taking advantage of the observation that the GLP-1 receptor is expressed at a high density in the islets, but not in exocrine cells (10). This approach has proven useful for imaging insulinomas (11), but has not yet been demonstrated to allow for the determination of islet cell mass in healthy humans or patients with diabetes. Harris and colleagues used [11C]DTBZ, a ligand to vesicular monoamine transporter 2 (VMAT2), to image pancreatic islets in rodents and humans (12–14) by the means of positron emission tomography (PET). Although initial results with DTBZ have been rather encouraging (12,13), recent findings suggest that nonspecific, non–β-cell binding of [11C]DTBZ in the pancreas may limit its utility as a β-cell imaging agent in humans (14).

An obvious reason for these difficulties in generating a valuable imaging agent is the paucity of knowledge about potential targets that are exclusively expressed on the β-cell surface. One way to overcome these limitations is to use phage-display technology, a powerful approach for isolating target-specific peptides and antibodies in animals and humans (15,16). Such single-chain antibodies (SCAs)
that trigger receptor endocytosis directly have previously been isolated by recovering infectious phages from within cells after receptor-mediated endocytosis (17). For example, internalizing SCAs targeting erbB2 and epidermal growth factor receptor have been identified and used to specifically deliver drugs into breast cancer cells (18,19). For in vivo screening, directly isolated rat islets were incubated with \(10^7\) phage transducing units for 1 h at 37°C in vitro, as described previously (21). Subsequently, islets from both approaches were treated equally to rescue phages from within the islet cells. Briefly, the islets were washed twice by suspension in 1 ml PBS, followed by lysing the cells by the addition of 1 ml hypotonic solution (30 mmol/l Tris-HCl, pH 8.0) and a single freeze–thaw cycle. Subsequently, the suspension was incubated overnight with TG1 bacteria at 37°C, with transducing units determined to monitor the progress of each round of library panning and the phages amplified according to standard protocols for use in the next round (22).

**Purification of SCAs.** HB2151 bacteria (OD\(_{600}\) 0.4) were infected with \(10^{12}\) phage clones per ml and the suspension was heated in the microwave antigen unmasking solution pH 6 and a single freeze–thaw cycle. Subsequently, the suspension was incubated overnight with TG1 bacteria at 37°C, with transducing units determined to monitor the progress of each round of library panning and the phages amplified according to standard protocols for use in the next round (22). **Purification of SCAs.** HB2151 bacteria (OD\(_{600}\) 0.4) were infected with \(10^{12}\) phage clones per ml and the suspension was heated in the microwave antigen unmasking solution pH 6 and a single freeze–thaw cycle. Subsequently, the suspension was incubated overnight with TG1 bacteria at 37°C, with transducing units determined to monitor the progress of each round of library panning and the phages amplified according to standard protocols for use in the next round (22).

**Immunohistochemistry.** Staining of formalin-fixed, paraffin-embedded rat and human tissue sections (5 \(\mu\)m) was performed as follows: Sections were deparaffinized using xylol twice for 10 min and followed by EtOH thrice for 5 min and Aquadest for another 5 min. Afterward, the sections were permeabilized by heating in the microwave in antigen unmasking solution pH 6 and a single freeze–thaw cycle. Subsequently, the suspension was incubated overnight with TG1 bacteria at 37°C, with transducing units determined to monitor the progress of each round of library panning and the phages amplified according to standard protocols for use in the next round (22).

**Phage screening.** The library was screened for islet-specific SCAs using two approaches. 1) For in vivo screening, a nonadherent CD-rat was injected intravenously through the jugular vein with \(10^7\) phage transducing units and allowed to circulate for 5 min. The pancreas was removed, and to recover islet bound phages, the islets were isolated according to a standard protocol except the use of FCS (20). 2) For in vitro screening, directly isolated rat islets were incubated with \(10^7\) phage transducing units for 1 h at 37°C in vitro, as described previously (21). Subsequently, islets from both approaches were treated equally to rescue phages from within the islet cells. Briefly, the islets were washed twice by suspension in 1 ml PBS, followed by lysing the cells by the addition of 1 ml hypotonic solution (30 mmol/l Tris-HCl, pH 8.0) and a single freeze–thaw cycle. Subsequently, the suspension was incubated overnight with TG1 bacteria at 37°C, with transducing units determined to monitor the progress of each round of library panning and the phages amplified according to standard protocols for use in the next round (22).

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The following day, the \( ^{125} \text{I} \)-SCA B1 (each animal got 0.002 \( \mu \)g) was injected intravenously in these animals. Two hours after injection, the fasting plasma glucose \([\text{FPG}]\): 250 \( \pm \)10 mg/dl), high-dose STZ \((n = 5)\), fasting plasma glucose \([\text{FPG}]\): 400 \( \pm \)97 mg/dl), and nondiabetic control rats \((n = 7)\). The \( ^{125} \text{I} \)-SCA B1 (each animal got 0.002 \( \mu \)g) was injected intravenously in these animals. Two hours after injection, the animals were killed, and pancreases were removed, weighed, and assayed in the gamma counter for radioactivity. \( ^{125} \text{I} \) was labeled with \(^{125} \text{I} \)-SCA B1, IPGTTs, and estimation of \( \beta \)-cell mass. In this set of experiments, an IPGTT was performed 14 days after diabetes induction (see above for details of these procedures, except that insulin and glucagon levels were not determined) in low-dose STZ \((n = 5)\), fasting plasma glucose \([\text{FPG}]\): 250 \( \pm \)10 mg/dl), high-dose STZ \((n = 5)\), fasting plasma glucose \([\text{FPG}]\): 400 \( \pm \)97 mg/dl), and nondiabetic control rats \((n = 7)\). The \( ^{125} \text{I} \)-SCA B1 (each animal got 0.002 \( \mu \)g of body wt) was injected intravenously in these animals. Two hours after injection, the animals were killed, and pancreases were removed, weighed, and assayed in a gamma counter for radioactivity. Accumulation of SCA B1 was expressed as a %ID/g of tissue, corrected for background, and estimated in the pancreas (milligram)/H11005/H9252 fraction (%). The \( \beta \)-cell mass per pancreas (milligram) = \( \beta \)-cell fraction \( \times \) pancreatic weight (milligram).

**Statistics.** Curve fits were modeled in form of nonlinear regression. For washout experiments, data were adapted to an exponential decay function with \( y = a + b \text{exp}(-x^c) \), where \( x \) denotes the time axis and \( y \) the measured count rate. For saturation experiments, results were adapted to an exponential rise function \( y = y_0 + b(1 - \text{exp}(-c x)) \). Parametric comparisons of continuous data were calculated with Student’s \( t \) test for unpaired data with unequal variance. Main null hypothesis was equal distribution of measured counts in both investigated cell types or with and without preincubation, respectively. Area under the curve (AUC) for glucose, insulin, and glucagon was calculated using the trapezoidal rule.

**Radioactive in vitro assay.** The assay was performed as described previously, except that an electronic pulse area analysis (CASY Technology, Reutlingen, Germany) was used to estimate the average cell volume (fl). To evaluate specificity of binding, SCAs were preincubated with selected unlabeled SCAs (20 \( \mu \)g) for competition assays.

**Pharmacokinetic analysis.** Rats injected with 100 \( \mu \)g radiolabeled SCAs were killed at indicated time points, and blood samples were obtained, followed by sedimentation of cellular material and precipitation of supernatant with trichloroacetic acid. Radioactivity associated with pellets and supernatant was measured. Blood content of radiolabel was expressed as a percentage of injected dose per gram of blood (%ID/g).

**Intraperitoneal glucose tolerance test.** CD-rats received an intraperitoneal glucose tolerance test (IPGTT) 7 days after intravenous injection of SCAs.

**Cell viability and apoptosis.** INS-1 (kind gift from C. Wollheim, Geneva), AR42J (ATCC, Manassas, VA), and \( \alpha \)-TC1 cells (ATCC, Manassas, VA) were grown (23,24) and processed as described (23).

**Imaging agents selective to pancreatic \( \beta \)-cells.**

![Image](https://example.com/image.png)

**FIG. 2.** Immunofluorescence analyses of binding selectivity to pancreatic islets after intravenous application of ISPCs, a control ISPC without an insert, or SCAs in a rat \((n = 4)\) rats per group, 30–40 sections and 60–80 islets per rat). Double staining of ISPCs \((A–F)\) and of SCA A1 in \( \beta \)-cells \((G–J)\) was not detectable in the islets. In another set of experiments, double staining of SCAs \((G–J)\) and of glucagon \((H, I, red)\) and nucleus using DAPI (blue). The ISPC1 \((A and B)\) colocalized exclusively with insulin, whereas the ISPC3 \((C and D)\) colocalized selectively with glucagon. In contrast, the control ISPC without an insert \((E and F)\) was not detectable in the islets. In another set of experiments, double staining of SCAs \((G–J)\) and of glucagon \((H, I, red)\) and nucleus using DAPI (blue) was performed. This confirmed the highly selective uptake of SCA B1 in \( \beta \)-cells \((G and H)\) and of SCA A1 in \( \alpha \)-cells \((I and J)\). All images were acquired at magnification \( \times 40 \). (A high-quality digital representation of this figure is available in the online issue.)
RESULTS

Generation of SCAs binding selectively to either β- or α-cells in rats. To generate agents specifically binding to pancreatic islets, a phage library was screened for SCAs on rat islets using two different approaches: A: The library was injected into rats in vivo, and islets were isolated after a circulation time of 5 min. B: Pancreatic islets were directly isolated, and the library was panned in the islets in vitro. After five rounds of selection, a marked increase in the phage transducing units per islet over successive rounds of panning was found, demonstrating a 700- and 500-fold enrichment with approach A and B, respectively. Subsequently, the DNA encoding the corresponding phage-displayed SCAs was sequenced. By these means, four islet-specific phage clones (termed ISPC1 = SCA B1, ISPC2 = SCA B2, ISPC3 = SCA A1, ISPC4 = SCA A2) were identified by approach A (Fig. 1). Approach B also yielded four ISPCs, two of which were identical to those derived from approach A (ISPC1 and 2). The other two ISPCs were termed ISPC5 and 6 (SCA B3 and SCA B4).

Binding selectivity and subcellular localization of SCAs. To determine the binding selectivity of the ISPCs to rat islets in vivo, the ISPCs were injected intravenously and allowed to circulate for 2 h. Subsequently, the animals were killed and the pancreas and control organs were harvested and prepared for immunohistochemical analyses. Immunostaining for ISPC1 was readily detectable in the cytoplasm of islet cells, where it was colocalized to insulin, but not glucagon staining.
suggesting selective binding to \( /H9252\) cells (Fig. 2A and B). Similar staining patterns were found for ISPC2, 5, and 6 (data not shown). In contrast, ISPC3 (Fig. 2C and D) and 4 (data not show) were found to be exclusively colocalized with glucagon staining, indicating that these ISPCs were selectively binding to islet \(\alpha\)-cells. Of note, no binding to exocrine cells (Fig. 2A–D) and several other control organs (liver, kidney, spleen, heart, and lung) was detectable in any of the ISPCs (Fig. 3). Furthermore, control experiments using an ISPC without the insert did not reveal any binding activity to pancreatic islets or other structures (Fig. 2E and F).

Subsequently, soluble SCAs containing a c-Myc tag and a His\(_6\) tag were generated from all six ISPCs in small-scale cultures. These SCAs were then purified by metal affinity chromatography and administered intravenously into rats. These experiments confirmed the highly selective cytoplasmatic uptake of SCA B1 (Fig. 2G and H) and B2–4 (data not shown) in \(\beta\)-cells and of SCA A1 (Fig. 2I and J) and A2 (data not shown) in islet \(\alpha\)-cells, whereas no

FIG. 5. Analyses of the binding process of selected \([^{125}\text{I}]\)-labeled SCAs to different endocrine and exocrine cell lines in vitro and determination of their pharmacokinetic profiles in vivo. Binding specificity of SCA B1 to INS-1 cells (A, \(P = 0.0016\) vs. \(\alpha\)-TC1 or AR42J) and SCA A1 to \(\alpha\)-TC1 cells (B, \(P < 0.0001\) vs. INS-1 or AR42J). Time course of binding of SCA B1 to INS-1 cells (C, \(t_{1/2} = 8.0\) min) or SCA A1 to \(\alpha\)-TC1 cells (D, \(t_{1/2} = 5.3\) min). Competition assay of SCA B1 (E, \(P < 0.0001\) vs. preincubation (PI) with SCA B1) or SCA A1 (F, \(P < 0.0001\) vs. preincubation with SCA A1) with unlabeled SCAs. Dose response of SCA B1 binding to INS-1 cells (G, \(r^2 = 0.96\)) or SCA A1 to \(\alpha\)-TC1 cells (H, \(r^2 = 0.96\)). Time course of elimination of SCA B1 (I, \(t_{1/2} = 22.7\) min, \(r^2 = 0.87\)) or SCA A1 (J, \(t_{1/2} = 19.2\) min, \(r^2 = 0.97\)) from the vascular system. Error bars represent SEM. CAR, cell-associated radioactivity.
binding to exocrine cells (Fig. 2G–J) and other control tissues (liver, kidney, spleen, heart, and lung) was detected for any of the SCAs (Fig. 3).

Finally, the SCAs were applied for electron microscopy in rat pancreatic tissue. These analyses confirmed the selective cytoplasmatic localization of SCA B1 and SCA A1 for islet β-cells (Fig. 4A and B) and α-cells (Fig. 4C and D), respectively. More specifically, selective binding to the endoplasmatic reticulum and the secretory granules of the respective target cells was found.

**Binding properties of radiolabeled SCAs in vitro and pharmacokinetic profiles in vivo.** To determine the respective binding properties of selected ¹²⁵I-labeled SCAs and to compare these characteristics with other agents examined previously, a systematic in vitro analysis was performed in the β-cell line INS-1, the α-cell line α-TC1, and the exocrine cell line AR42J. Binding of the β-cell–specific SCA B1 to INS-1 cells was 514 times higher than binding to α-TC1 and AR42J cells (Fig. 5A). In contrast, the α-cell–specific SCA A1 showed a 210-fold higher binding specificity to α-TC1 relative to INS-1 and AR42J cells (Fig. 5B), consistent with the in vivo findings. Furthermore, rapid binding characteristics were found for both SCAs, with a binding half-life of 8.0 min for SCA B1 in INS-1 cells (Fig. 5C) and a binding half-life of 5.3 min for SCA A1 in α-TC1 cells (Fig. 5D).
Competition assays demonstrated no binding inhibition of radiolabeled SCA B1 by preincubation with unlabeled SCA A1 and vice versa (Fig. 5E and F), suggesting that the binding and internalization of these SCAs were mediated by different molecules. For both SCAs, binding saturation could be demonstrated after preincubation with the corresponding unlabeled SCAs (Fig. 5E and F). The respective dose-response curves revealed a maximum capacity of 650.307 SCA B1 molecules to be internalized per H9252-cell and of 669.945 SCA A1 molecules to be taken up per α-cell (Fig. 5G and H).

Because any unbound label circulating in the vasculature might theoretically interfere with the respective imaging signal of the bound label, the plasma clearance kinetics of selected SCAs were determined in rats in vivo (Fig. 5I and J). For these purposes, preparations of radiolabeled SCAs were administered intravenously into rats, and the respective radioactivity was measured in the plasma over time. These experiments revealed a very rapid elimination of both SCA B1 and SCA A1 from the circulation ($t_{1/2} = 22.7$ min and 19.2 min, respectively).

**Specific binding to human islets in situ.** To use these SCAs for the determination of islet cell mass in humans, specific binding to human pancreatic β- or α-cells would be required. Therefore, human pancreatic tissue was costained by immunohistochemistry using the SCAs as well as specific antibodies for insulin and glucagon, respectively. Staining for the β-cell–specific SCA B1 was clearly detectable within the islets and colocalized with insulin expression, whereas no binding to α-cells or exocrine cells was detectable (Fig. 6A and B). Conversely, the α-cell–specific SCA A1 overlapped with glucagon staining, but neither with insulin staining nor with exocrine cells (Fig. 6C and D).

**In vivo toxicity tests.** Because any interference of the SCAs with islet cell function would clearly limit their clinical applicability, IPGTTs were carried out 7 days after the intravenous administration of the SCAs into rats. The time course of plasma glucose, insulin, and glucagon levels during the IPGTT was unchanged by the SCAs compared with vehicle-treated animals (Fig. 7A–C), suggesting no impairment in islet function. Furthermore, to rule out direct toxic effects on islet cell turnover, cell viability (Fig. 7D) and apoptosis (Fig. 7E) were assessed in INS-1 or α-TC1 cells after overnight exposure to increasing amounts of the SCAs in vitro. No influence of islet cell turnover was found in these experiments.

**Quantification of β-cell mass using [$^{125}$I]-SCA B1 in rats.** To test the ability of SCA B1 for the quantification of β-cell mass in vivo, 14 days after diabetes induction by STZ treatment, the [$^{125}$I]-SCA B1 was administered intravenously into rats and allowed to circulate for 2 h. Subsequently, the animals were killed, and the accumulation of
the $[^{125}\text{I}]$-SCA B1 was determined in the harvested pancreas. The measured radiolabeling intensity in the pancreas was then compared with the respective $\beta$-cell mass (determined by quantitative morphometry) and the glucose excursions (AUC glucose) during an IPGTT (performed the day before $[^{125}\text{I}]$-SCA B1 application).

Both $\beta$-cell mass and the respective radiolabeling intensity were higher in nondiabetic rats than in low- and high-dose STZ-injected diabetic rats (Fig. 8A). A strong positive correlation between the radiolabeling intensity of the pancreas and $\beta$-cell mass was found within all three subgroups ($r^2 = 0.937$), with the respective probe accumulation diminishing gradually with declining $\beta$-cell mass. Furthermore, an inverse relationship between radiolabeling intensity and glucose excursions during the IPGTT was observed ($r^2 = 0.876$, Fig. 8B).
DISCUSSION

We report the successful isolation of six SCAs highly specific for either β- or α-cells both in rodents and in humans. Furthermore, a direct correlation between pancreatic uptake of radiolabeled SCAs and β-cell mass was observed in normal and diabetic animals.

Antibodies have been used for cell-specific delivery of therapeutic compounds, e.g., tumor-specific delivery of chemotherapeutics (28). Therefore, it is reasonable to suppose that antibodies directed against a highly specific β-cell molecule would be of clinical utility for imaging the β-cell mass. In this context, Moore et al. (26) used a [111In]-labeled IC2 antibody, directed against an unknown molecule, which showed excellent correlation between probe accumulation and β-cell mass ex vivo. However, because of the IgM nature of the antibody, a very slow excretion from the vasculature (several days) was evident, leading to an unfavorable signal-to-background ratio in vivo (29). In another study, Ladriere et al. (30) evaluated a [125I]-labeled mouse monoclonal antibody (R2D6) directed against a β-cell surface ganglioside. Although the binding of [125I]-R2D6 was higher in isolated islets than in actinaria tissue, no significant difference was detected when comparing islets from normal and rats rendered diabetic by STZ. Recently, Hampe et al. (23) tested another β-cell-specific IgM antibody (K14D10) and concluded that the specificity was far below that required to overcome the signal-to-background ratio in vivo. However, it has been reported that removal of the Fc portion to produce an antibody fragment or an SCA reduces blood clearance time and nonspecific binding (31,32). Therefore, antibody-mediated targeting of β-cells seems to be a promising approach; however, an antibody with excellent binding characteristics, but with a much faster clearance from the vasculature, would need to be developed.

Here, we report the application of a phage-panning approach in rodents to generate SCAs with highly specific binding to pancreatic β-cells in vivo. By these means, we succeeded to isolate four SCAs that are selectively internalized into rodent β-cells in vivo, home to the endoplasmatic reticulum and the insulin secretory granules, and bind to human β-cells in situ. Furthermore, two additional SCAs homing to islet α-cells were identified. These SCAs were highly specific for their respective target cells with no relevant binding to other islet cells, exocrine cells, or other tissues and with a calculated in vitro selectivity of >500:1 for β-cells and >200:1 for islet α-cells. These numbers are far in excess of these reported previously for other potential β-cell mass imaging compounds and meet the requisite exocrine-to-endocrine binding ratio needed to distinguish β-cells (8,9).

Although these studies cannot completely clarify the precise mechanisms subserving the rapid and high-volume (>650,000 SCAs/cell) binding and cellular uptake of SCAs to either β- or α-cells, a membrane receptor-mediated endocytosis appears to be most likely. Moreover, we propose that the intracellular localization of the antibodies has resulted from our screening strategy, which involved lysis and homogenization, meaning that the membrane-bound antibodies were likely eliminated, whereas the intracellular ones were rescued, amplified, and reinjected several times. Such mechanism would be consistent with previous studies in this area in other cell types (17–19,33,34) and would also be consistent with the competitive binding characteristics of different SCAs at β- and α-cells. However, although it seems highly likely that the intracellular uptake of the SCAs was indeed receptor mediated, the specific molecular target remains to be elucidated.

Another important characteristic of the presently described SCAs in favor of a diagnostic application in vivo is the avid elimination of the unbound particles from the circulation. These properties are in line with recent studies showing a rapid elimination of SCAs mainly via the kidneys and have been attributed to their rather low molecular weight (~31 kDa) (32,35).

In addition, this proof of concept study found no alteration of islet turnover or islet function by the respective SCAs. However, more detailed experiments will be required (dose-escalating studies in vivo, repeated applications in vivo, etc.) to completely rule out any toxicity or
antibody formation against the SCAs, before these novel SCAs can be safely applied to humans.

Finally, the SCA B1 tested herein has proven to not only label islet β-cells in vitro or in human pancreas in situ but also provide an excellent tracer for the radiolabeling of β-cells in vivo. Thus, the labeling intensity derived from the [18F]-SCA B1 after intravenous administration in rats in vivo strongly predicted the respective β-cell mass and was inversely related to the glucose excursions during an IPGTT, even though insulin secretion was not directly assessed in this study. Therefore, our data provide strong evidence that the SCAs characterized herein allow for the imaging and quantification of β-cell mass in vivo, which has to be confirmed in future studies using noninvasive imaging techniques such as PET.

In conclusion, we have generated SCAs that bind specifically to β- or α-cells of rodent or human origin and exhibit a high selectivity for their respective target cells over other tissues. These radiolabeled SCAs have proven to reliably predict β-cell mass in rodent models of diabetes after in vivo administration. We propose that such SCAs combined with less invasive imaging techniques, such as PET, may be suitable for the in vivo determination of β-cell mass in humans.

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S.S. and R.S. hold patent application for β-cell imagina. No other potential conflicts of interest relevant to this article were reported.

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