

Peptide-Mediated Targeting of the Islets of Langerhans

Kausar N. Samli,¹ Michael J. McGuire,¹ Christopher B. Newgard,² Stephen Albert Johnston,¹ and Kathlynn C. Brown¹

Strategies for restoring β -cell function in diabetic patients would be greatly aided by the ability to target genes, proteins, or small molecules specifically to these cells. Furthermore, the ability to direct imaging agents specifically to β -cells would facilitate diagnosis and monitoring of disease progression. To isolate ligands that can home to β -cells in vivo, we have panned a random phage-displayed 20-mer peptide library on freshly isolated rat islets. We have isolated two 20-mer peptides that bind to islets ex vivo. One of these peptides preferentially homes to the islets of Langerhans in a normal rat with clear differentiation between the endocrine and exocrine cells of the pancreas. Furthermore, this peptide does not target β -cells in a type 2 diabetes animal model, suggesting that the peptide can discriminate between glucose-stimulated insulin secretion-functional and -dysfunctional β -cells. *Diabetes* 54: 2103–2108, 2005

The β -cells of the islets of Langerhans in normal mammals adjust their secretion of insulin in response to changes in the levels of metabolic fuels and insulinotropic hormones. Loss of β -cell function is central to the development of both major forms of diabetes. Type 1 diabetes occurs when pancreatic islet β -cells are destroyed by the host immune system. Type 2 diabetes involves loss of key β -cell functions such as glucose-stimulated insulin secretion (GSIS) and a gradual loss of β -cell mass by nonautoimmune mechanisms. Intensive research efforts are currently focused on identifying pathways by which insulin secretion can be increased or β -cell growth and survival can be enhanced. However, the therapeutic relevance of such discoveries for enhancing islet function or preserving β -cell mass in pre-diabetic or diabetic states may be limited by the inability to deliver new therapeutic drugs, genes, or proteins directly to islet

β -cells. The current study attempts to address this issue by the application of phage-display technology for the identification of peptides that target specifically to β -cells in pancreatic islets of living animals.

Specific delivery of molecular cargo to pancreatic islets has been a long-standing goal of diabetes researchers. Recombinant adenovirus and adeno-associated virus vectors have been used for efficient delivery of genes and, more recently, small interfering RNA constructs to primary cultures of pancreatic islets (1–4). However, these vectors have traditionally not been useful for delivery of genes to pancreatic islets of living animals because of very efficient clearance of systemically injected virus by the liver and the need to traverse vascular barriers in islets. Recently, some limited success in gene transfer (an average of 20% of cells in 70% of islets) has been reported in studies involving systemic infusion of adenovirus into mice in which the hepatic circulation (portal vein, hepatic artery, and bile duct) had been surgically clamped for a period of 30 min (5). The major surgical intervention required in this approach and the potential for immune responses to adenoviral vectors may limit its applicability for human studies. Another recent study presented an alternative strategy for islet targeting involving infusion of a cationic peptide transduction domain (PTD-5) fused to peptide inhibitor of I κ B kinase (IKK), resulting in improved islet function and viability upon subsequent isolation (6). Implementation of this strategy required infusion of the PTD-5/IKK inhibitor fusion peptide via the surgically resected bile duct, again seemingly limiting its broad applicability. Clearly, strategies for restoring β -cell function and preserving islet mass in diabetes would be greatly aided by the ability to target genes, proteins, or small molecules in a more noninvasive and specific fashion than has been achieved to date. Furthermore, the ability to direct imaging agents specifically to β -cells would facilitate diagnosis and monitoring of disease progression (7).

M13 phage-display technology is a powerful approach for isolating peptides that bind to cell surface receptors (8). In vivo phage panning has been successfully used to isolate peptides that mediate targeting to the vasculature beds of specific organs (9,10), including the pancreas (11). However, no accumulation of these selected peptides has been observed in the islets. Targeting to islets is made challenging by the fact that these cells in aggregate represent only 2% by weight of the entire pancreas (12). We have previously demonstrated that phage panning can be carried out on cells in culture to obtain peptides that mediate specific binding to the target cells while not binding to undesired cell types (8,13–17). The high discriminating power of the selected phage suggests that peptides could be identified that specifically bind to β -cells

From the ¹Center for Biomedical Inventions, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; and the ²Sarah W. Stedman Nutrition and Metabolism Center, Departments of Pharmacology and Cancer Biology, Medicine, and Biochemistry, Duke University Medical Center, Durham, North Carolina.

Address correspondence and reprint requests to Kathlynn C. Brown, Center for Biomedical Inventions, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9185. E-mail: kathlynn.brown@utsouthwestern.edu.

Received for publication 24 January 2005 and accepted in revised form 11 April 2005.

Additional information for this article can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

GSIS, glucose-stimulated insulin secretion; HBSS, Hank's balanced salt solution; RIP, rat islet peptide.

© 2005 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

within the pancreas. Furthermore, since intact cells are used as the bait for peptide binding, no prior information about β -cell-specific cell surface biomarkers is required. Toward the eventual goal of delivering molecular cargo to β -cells in a specific fashion *in vivo*, we have panned a phage-displayed peptide library on isolated rat islets and have isolated a 20-amino acid peptide that homes to the islets of Langerhans within living rats.

RESEARCH DESIGN AND METHODS

Cell lines and reagents. A previously described random 20-mer peptide library, designated ON543.2, was used for the isolation of β -cell-specific peptides (18). This library represents 10^8 different peptide sequences displayed on the NH_2 -terminus of the pIII protein of filamentous bacteriophage fd-tet. The INS-1-derived INS 832/13 cell line was maintained in culture as previously described (19). Female Sprague-Dawley rats were purchased from Harlan Industries (Indianapolis, IN). Male Zucker diabetic fatty (ZDF) rats were purchased from Charles River Laboratories (Wilmington, MA) and fed Purina Mills Formulab Diet 5008 (St. Louis, MO) to maintain their diabetic state (20,21). The experiments were performed with 12-week-old ZDF rats, at which time the animals were diabetic. All animal protocols were approved by the institutional animal care and research advisory committee at the University of Texas Southwestern Medical Center, Dallas, Texas.

Isolation of primary islets. Primary islets were isolated from six female Sprague-Dawley rats weighing ~225–250 g for each panning experiment according to standard protocols with minor modifications (22,23). Hank's balanced salt solution (HBSS, pH 7.4; Invitrogen, Carlsbad, CA) supplemented with 1g/l of glucose and 200 mg/l BSA (Sigma-Aldrich, St. Louis, MO) was aerated with a 95% O_2 and 5% CO_2 gas mixture for 20 min on ice. The pancreas was perfused with 50 ml enzymatic digestion HBSS containing collagenase (112 units/ml; Sigma-Aldrich). Two pancreata at a time were treated by collagenase digestion at 37°C over a period of 21 min. During the first 5 min, the solution was aerated with 95% O_2 and 5% CO_2 gas, followed by continued incubation without the gas mixture for the next 4 min. The flask was vigorously shaken every 2 min for the remaining 12 min while being incubated. The digested pancreas was isolated by centrifugation, and discontinuous Ficoll (Amersham Biosciences, Piscataway, NJ) gradients in HBSS were used to separate islets. The samples were centrifuged at 500g for 20 min at 4°C. After collecting islets from all six rats, the islets were isolated by centrifugation at 200g and immediately used for panning.

Ex vivo phage library panning on primary rat islets. The panning procedure was modified from previously published protocols (8,14) for use with freshly isolated islets. Preclearing of cell surface receptors was not necessary since islets were isolated in serum-free media. Islets in suspension were mixed with 3×10^{10} phage particles, 100 $\mu\text{mol/l}$ chloroquine (Sigma-Aldrich), and $1 \times$ protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Islets were incubated with phage for 1 h at 37°C with gentle shaking. The islets were washed twice by suspension in 1 ml HBSS, followed by one 1-ml wash with 0.1 N HCl-Glycine (pH 2.2). The cells were lysed by the addition of 1 ml hypotonic solution (30 mmol/l Tris-HCl, pH 8.0), followed by a single freeze-thaw cycle. The phage from the output fraction was amplified according to standard protocols for use in the next round of panning (24). Aliquots of the initial input and output samples were titered, and the output-to-input phage ratio based on colony-forming units was determined to monitor the progress of each round of library panning (24). To compare phage binding to cells, an output-over-input ratio was calculated as follows: output-to-input ratio = output titer of phage from lysed cells/input titer of total phage incubated with cells.

Sequence determination. Random phage clones from titration plates were routinely sequenced after round 3 of the library panning to identify convergent peptide sequences as previously described (15). Convergent peptide-displaying phage clones were isolated for further characterization.

In vitro phage specificity panning. Isolated phage clones selected from the library panning were characterized by panning on cell lines as previously described (14) using 2×10^8 phage particles of each phage clone. Incubations were carried out for 1 h before the removal of unbound and surface-bound phages. Specificity panning on islets was performed in the same manner as the library panning with the exception that 2×10^8 phage particles of a single-phage clone were placed on the purified islets from Sprague-Dawley rats. Colony-forming units were determined from bacterial titrating of the output and input samples, and output-to-input ratios were calculated to determine phage binding.

In vivo phage delivery in Sprague-Dawley and ZDF rats. Rats were anesthetized with pentobarbital at 100 mg/kg and injected with 2×10^8 phage

particles via the jugular vein. After 2 h, the animals were perfused with 150 ml PBS via cannulation of the carotid artery. The abdominal cavity was opened to expose the liver to monitor blanching of this tissue during perfusion. Heart, lung, spleen, pancreas, skeletal muscle, kidney, and liver were harvested and fixed in Carson's modified buffered formalin (Richard-Allan Scientific, Kalamazoo, MI) overnight. Paraffin processing, embedding, and sectioning were performed by standard procedures by the Molecular Pathology Core at the University of Texas Southwestern Medical Center.

Immunohistochemistry. Paraffin-embedded tissue sections were deparaffinized using a Sakura Tissue-Tek DRS (Torrence, CA). Tissue samples were permeabilized with 0.5% Triton X-100 in PBS for 5 min at room temperature followed by blocking with 2% normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA) in PBS⁺ (8.2 mmol/l Na_2PO_4 , 1.5 mmol/l KH_2PO_4 , 137 mmol/l NaCl, 2.7 mmol/l KCl, and 0.5 mmol/l CaCl_2 , pH 7.4) containing 0.5% BSA for 40 min. Spleen samples were also blocked using FC-Block (1:200) (BD Pharmingen, San Diego, CA) to minimize non-antigen-specific binding. The samples were incubated overnight at 4°C with a 1:50 dilution of mouse anti-phage polyclonal antibody (15) or mouse monoclonal anti-insulin antibody (Sigma, St. Louis, MO) in PBS⁺/0.5% BSA. Excess antibody was washed off with PBS⁺ containing 0.5% BSA and a 1:24 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin antibody (Jackson ImmunoResearch Laboratories) was incubated with the tissues for 1 h at room temperature. Excess antibody was removed with serial washes with PBS, and a 1:5,000 dilution of Hoechst Dye #33342 (Molecular Probes, Eugene, OR) was used to stain nuclei. Tissue samples were covered with Vectashield (Vector Laboratories, Burlingame, CA). Tissue slides were evaluated and photographed via fluorescent microscopy on an Eclipse TE2000 fluorescent microscope (Nikon, Melville, NY) equipped with a CoolSNAP_{fx} camera (Roper Scientific, Tucson, AZ). Images were captured using MetaVue v4.6r9 (Universal Imaging, Downingtown, PA) acquisition and analysis software.

RESULTS

Isolation of an islet-binding peptide from a peptide library. To obtain peptides that recognize β -cells in a biologically relevant system, freshly isolated pancreatic islets were used to pan a complex peptide phage library. This library contains 10^8 different 20-mer peptides fused to the NH_2 -terminus of the pIII coat protein of fd-tet bacteriophage. After six rounds of panning, a dramatic increase was observed in the output-to-input phage ratio, representing a 70-fold enrichment from the starting library (Fig. 1). Sequencing of 32 phage clones at round 6 of the panning revealed two major peptides. The first peptide, named rat islet peptide (RIP)-1, has the sequence LSGTPERS-GQAVKVKLKAIP and was present in 15 of the 32 clones. The second peptide, RIP2, represented 25% of the phage clones and has the sequence GAWEAVRDRIAEGWSW-GIPS. Sequencing of phage clones from earlier rounds of the panning process revealed that the RIP1 phage clone was present as early as round 4 of the panning protocol and was >20% of the phage pool at round 5. Surprisingly, the RIP2 sequence was not present until round 5 and then only as a single clone of the 28 sequenced. One other phage was present in duplicate in round 6, but because it represented <10% of the phage population and was not observed in earlier rounds of panning, this peptide was not retained for further analysis.

A BLAST (basic local assignment search tool) search of the National Center for Biotechnology Information sequence database for short, nearly exact matches of the RIP1 and RIP2 sequences encountered no significant homology with any known protein sequences (25).

RIP1 and RIP2 peptides mediate binding to islets. The binding of the RIP1 and RIP2 phage clones was tested on isolated islets, and the output-to-input phage ratio was used as a measure of phage binding to the islets. Both phage clones exhibit significant binding with 0.5% of the RIP1 phage and 7% of the RIP2 phage input being retained

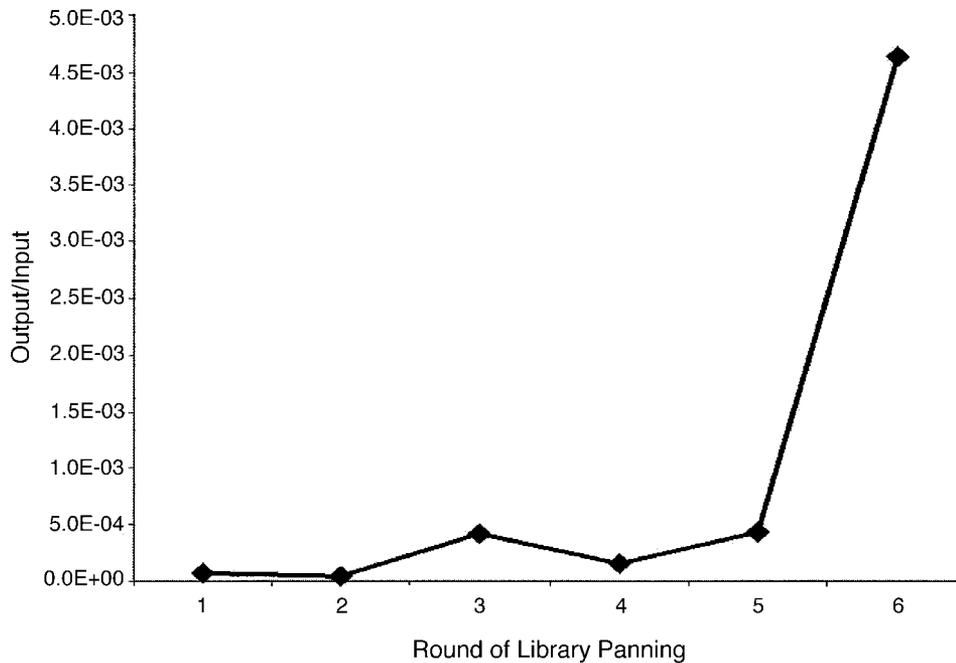


FIG. 1. The output-to-input ratio of bacteriophage increases over successive rounds of panning as binding phage clones are successively amplified and nonbinding phage clones are depleted. Binding of phage clones from round 6 is 70-fold greater than the complete library from round 1. Sequence results from round 6 indicate that RIP1 phage clone comprises 50% of the population and RIP2 comprises 25% of the population.

(Table 1). It is unexpected that the binding retention would be less for the RIP1 phage, which was the predominant clone in the phage selection. However, selection pressures other than cell binding can influence the isolation of phage clones, stressing the importance of assessing binding *in vitro* and *in vivo* of individual phage clones.

Since islets represent a mixture of cells, the peptides were tested for binding to a β -cell line in culture. Specifically, the output-to-input ratio of the two phage clones was determined for binding to cell line 832/13 (19), a cell line with robust GSIS derived from INS-1 rat insulinoma cells (26) (Table 1). Surprisingly, while the RIP2 clone has higher phage retention on the islets than RIP1, the converse is true for binding on the Ins832/13 β -cell line; eight times greater phage capture is observed for the RIP1 clone compared with the RIP2 phage. These data suggest that the RIP1 phage is binding to β -cells where the RIP2 phage may be binding to other cell types within the islets. It is important to note that both phage clones exhibit a significant decrease in the output-to-input phage ratio when studied on the β -cell line compared with primary islets. This decrease in phage capture could be the result of the cells in culture not being an ideal representation of β -cells *in vivo*, at least at the level of cell surface architecture. Alternatively, the discrepancy could arise from the difference in the total cell number of β -cells per islet compared with the number of β -cells in cultures that were used for panning.

The RIP1 phage homes to the islets of Langerhans in living rats. The true test of the relevance and potential

TABLE 1
Phage retention on isolated islets and a β -cell

Cell type	RIP1 phage output-to-input ratio	RIP2 phage output-to-input ratio
Primary islets	5.2×10^{-3}	7.3×10^{-2}
Ins832/13 β -cell line	2.1×10^{-4}	2.6×10^{-5}

The same phage amounts were included in all experiments.

applicability of our approach is to assess the ability of selected phage to target β -cells in an animal. To test this, RIP1, RIP2, or a control phage containing a random peptide sequence were injected into the jugular vein of a rat. Phage was allowed to circulate for 2 h, after which the animal was killed and organs harvested. The presence of phage in islets and other tissues was then determined by immunohistochemistry using an anti-phage antibody. The results are shown in Fig. 2. In animals that received the RIP1 phage, phage immunostaining is clearly identifiable in the islets and the signal overlaps with insulin expression, suggesting that the phage is binding to the β -cells. Diffuse, less intense staining is observed in the exocrine cells in the pancreas. Similar diffuse staining is seen throughout the pancreas of animals that have not received a phage injection or have been administered an irrelevant control phage, suggesting that this is background binding of antibody. However, we cannot rule out that the phage is also binding weakly to the nonendocrine cells of the pancreas. Similar but less dramatic preferential staining of the islets relative to the exocrine pancreas was obtained with the RIP2 peptide. Importantly, animals that were infused with the control phage containing a random peptide sequence exhibited no preferential phage immunolocalization in islets, indicating that the selected RIP1 and RIP2 peptides are mediating binding to the β -cells and this staining pattern is not due to nonspecific uptake of the phage particles (online appendix [available at <http://diabetes.diabetesjournals.org>]).

In order for the β -cell-targeting phage to be useful for further research, the peptides must localize within the islets but avoid uptake by nontargeted tissues. The binding of the RIP1 and RIP2 phage clones to other organs was characterized by performing immunohistochemistry using an anti-phage antibody (Fig. 3). The RIP2 phage clone demonstrates nonspecific accumulation throughout the animal, with especially strong accumulation occurring in the spleen, heart, and skeletal muscle (online appendix). However, no specific binding of RIP1 phage was observed

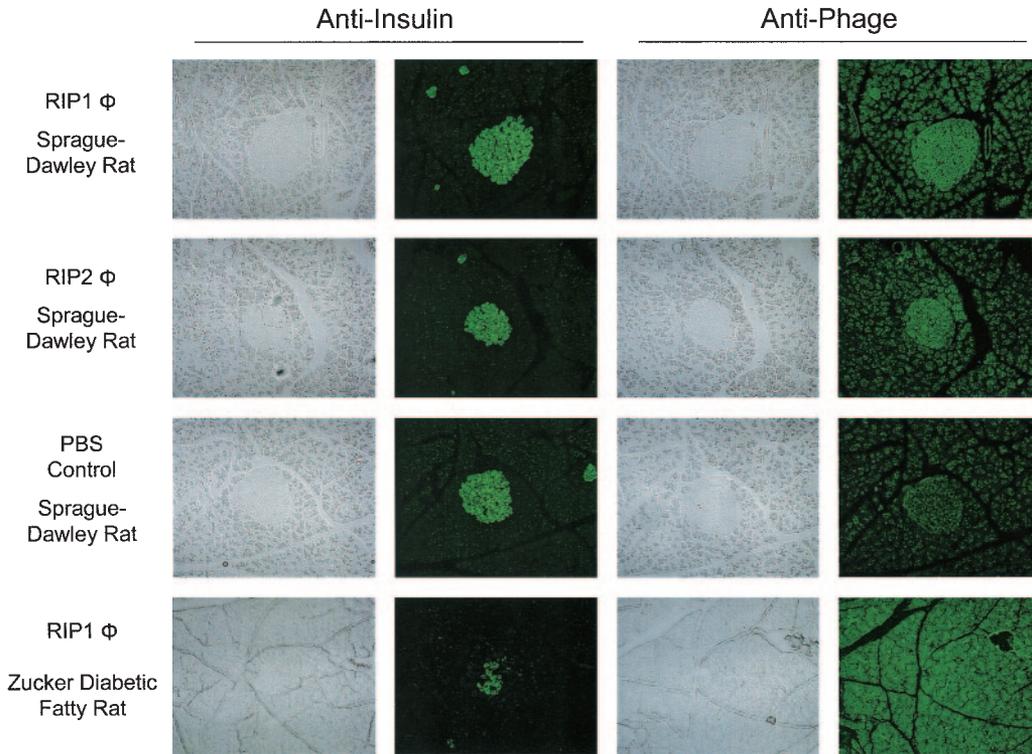


FIG. 2. In vivo distribution of RIP1 and RIP2 phage in pancreata from Sprague-Dawley or ZDF rats. The phage was injected intravenously into the jugular vein of a rat and allowed to circulate for 2 h, after which the animal was killed and organs harvested. The presence of the phage was determined by immunohistochemistry using an anti-phage antibody. Aligned sections are shown staining with anti-insulin or anti-phage antibodies. All panels are at 100× magnification. Significant accumulation of RIP1 and RIP2 phage in the islets of the Sprague-Dawley rats is visible. No significant accumulation of RIP1 phage is seen within the islet of a ZDF rat.

to heart, muscle, lung, or spleen tissue. Whereas some diffuse staining is observed in the liver of rats infused with RIP1, similar staining is seen with the control phage containing a random peptide sequence (online appendix) consistent with nonspecific clearance of the phage by the reticuloendothelial system. Similar results have been observed by our laboratory (15) and others (9,11). Interestingly, animals infused with the RIP1 phage exhibit significant staining in the kidney tubules. This is surprising because the size of the phage should prevent clearance through the kidneys. We suspect that the staining is a result of the clearance of degraded phage coat proteins

that are still recognized by the anti-phage antibodies. Similar intensity and staining patterns are observed in the kidneys from rats injected with all phage clones tested, indicating that the finding is unlikely to be explained by peptide-mediated binding in the kidneys. Overall, these data indicate that the RIP1 peptide is accumulating preferentially within the islets, although at this time, we cannot rule out the possibility that the peptide has some affinity for cells in the liver and kidney.

A valuable feature of β -cell-targeting reagents would be the ability to distinguish between normal, functional β -cells and those beginning to exhibit defective function,

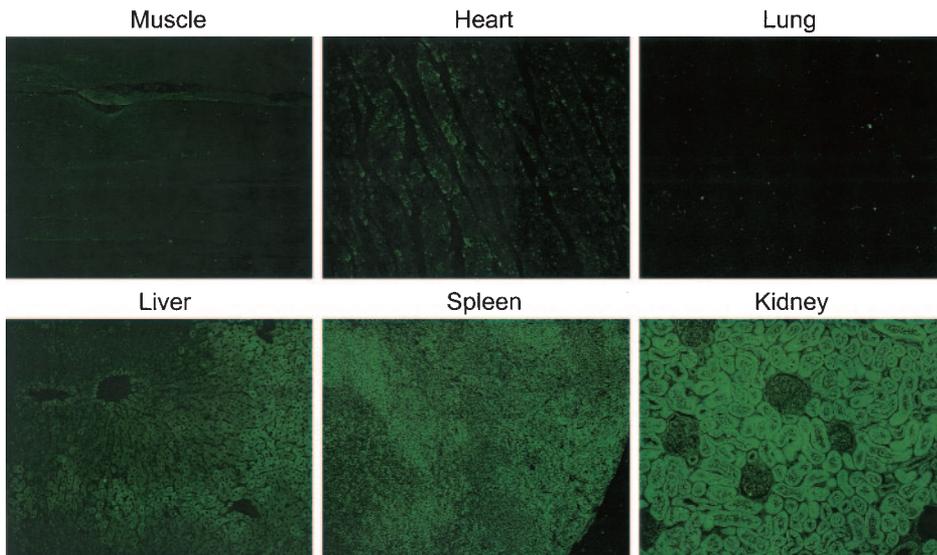


FIG. 3. In vivo distribution of the RIP1 phage throughout a Sprague-Dawley rat. No significant phage accumulation is observed in muscle, heart, and lung. Nonspecific clearance of phage can be seen in liver, spleen, and kidneys. All panels are at 100× magnification.

e.g., loss of GSIS. For this reason, we assessed the ability of the RIP1 phage to target β -cells in 12-week-old ZDF rats, an animal model of type 2 diabetes in which GSIS is dramatically impaired (20,21). As in the previous experiments, the phage was injected intravenously into the jugular vein of the rat. The phage was allowed to circulate for 2 h, after which the animal was killed and organs harvested. The presence of the phage was determined by immunohistochemistry using an anti-phage antibody. As shown in Fig. 2, the RIP1 phage has a very different staining pattern in the ZDF rats compared with the Sprague-Dawley rats. No specific accumulation of phage is observed in the islets and the intensity of phage staining is comparable to a control phage (online appendix). Taken together, these data suggest that the isolated RIP1 phage binds with specificity to fully functional pancreatic islets and loses its ability to target structurally disorganized and dysfunctional islets of ZDF rats.

DISCUSSION

The ability to target genes and therapeutics specifically to β -cells would facilitate diabetes treatment. Furthermore, determination of β -cell mass and function in vivo has been hampered by the inability to direct imaging agents to β -cells within the pancreas. Typically, cell-specific delivery is achieved by attaching the desired biomolecule to a ligand for a well-characterized cell surface molecule that is preferentially expressed in the targeted cell type. Using antibodies as the homing agent, this approach has been used for tumor-specific delivery of chemotherapeutics (27,28), and several immunotherapies are currently in the clinic (29). However, little is known about the cell surface profile of β -cells, and currently, the IC2 antibody is the only ligand used for delivery to β -cells in animals (30,31).

To circumvent the need for a defined biomarker, we used biopanning to isolate peptides that bind to islets of Langerhans from normal Sprague-Dawley rats. After six rounds of reiterative panning, the output-to-input phage ratio increased 70-fold. From a phage-displayed peptide library representing 10^8 different members, we isolated two 20-amino acid peptides, RIP1 and RIP2, that bind to islets *ex vivo* as determined by phage retention. It is important to note that little similarity is observed between the RIP1 and RIP2 peptide sequences, suggesting that they are most likely binding to different cellular receptors or different binding pockets within the same receptor. Furthermore, neither of these peptides show significant sequence similarity to previously isolated peptides that home to the pancreas vasculature (11). A BLAST analysis for short peptides of similar sequence revealed no significant homologies to known proteins in the NCBI database (25).

The major goal of this study was to identify peptides that mediate binding to β -cells within a living animal. Realization of this goal requires that the identified peptides have a significantly higher affinity for β -cells than for other cell types, the phage clones can escape the vasculature to reach the appropriate cells, and the peptide is stable in vivo. To address this, the isolated RIP1 and RIP2 phage clones were injected into normal Sprague-Dawley rats, followed by harvesting of tissue and immunohistochemical analysis with anti-phage antibodies to determine the phage localization. The RIP1 phage preferentially homes

to the islets of Langerhans in a normal rat with differentiation between the endocrine and exocrine cells of the pancreas. The immunohistochemical signal is observed throughout the islet and colocalizes with staining obtained with anti-insulin antibodies, indicating that the phage clone is binding to the β -cells within the islets. The RIP1 phage is not enriched in other tissues, although a nonspecific signal is observed in liver and kidney that is equal in intensity to that observed following injection of a control phage containing a random peptide sequence. We suspect that this signal is due to nonspecific clearance of the phage, but at this time, we cannot rule out that the peptide has some affinity for these cells as well. The RIP2 phage also accumulates in the islets, but the preferential localization to endocrine cells is less pronounced than observed with the RIP1 phage, even though the RIP2 displayed higher phage retention on islets *in vitro*. Furthermore, this phage showed significant accumulation in tissues and organs, indicating that this peptide binds nonspecifically to different cell types.

Because the RIP1 phage was selected on islets from healthy Sprague-Dawley rats, we sought to determine whether this phage clone could also target to β -cells in ZDF rats at 12 weeks of age, at which time the animals are severely diabetic and exhibit defective GSIS. We found that the RIP1 phage does not target β -cells in diabetic ZDF rats. This suggests that this peptide is recognizing a cell surface feature on normal, fully functional β -cells that is lost or obscured during disease progression. This cell surface feature might be related to the loss of GSIS, or alternatively, may be influenced by the increased infiltration of fibrotic tissue or the increased rate of cell death that occurs in ZDF islets in concert with progression to diabetes (32,33). Further studies will be required to determine whether RIP1 phage are able to target ZDF islets before onset of diabetes and conversely whether peptides can be identified that bind specifically to islets in diabetic animals.

In summary, we have isolated a peptide that is able to home to normal β -cells in the islets of Langerhans without prior knowledge of a β -cell biomarker. We anticipate that this peptide will be of utility for β -cell-specific delivery of molecular cargo, possibly including plasmids that contain specific therapeutic/protective genes, bioactive peptides or proteins, small molecules, or imaging agents. Use of the RIP1 peptide for these purposes will require demonstration of the ability of the isolated peptide, removed from the context of phages, to target islets in a manner similar to what has been observed for the phage clone. The peptide will then be utilized to deliver active biomolecules to the target cells. While these are significant hurdles, the current study provides promise for the use of selected peptides for islet-specific targeting applications in the near future.

ACKNOWLEDGMENTS

This work was supported by a Program Project Grant from the National Institutes of Health (5PO1 DK-05839805).

We thank Kimberly Jones-Ross for helpful experimental suggestions for islet isolation and Shuyuan Chen for technical expertise in establishing the *in vivo* delivery experiments. We also thank James A. Richardson, John M. Shelton, and the Molecular Pathology Core for their con-

tribution to the processing of tissue samples and slide preparation. Ya-Ching Chang and Chun Man Lee contributed technical assistance.

REFERENCES

1. Becker T, Beltrandel-Rio H, Noel RJ, Johnson JH, Newgard CB: Overexpression of hexokinase I in isolated islets of Langerhans via recombinant adenovirus: enhancement of glucose metabolism and insulin secretion at basal but not stimulatory glucose levels. *J Biol Chem* 269:21234–21238, 1994
2. Flotte T, Agarwal A, Wang J, Song S, Fenjves ES, Inverardi L, Chesnut K, Afione S, Loiler S, Wasserfall C, Kapturczak M, Ellis T, Nick H, Atkinson M: Efficient ex vivo transduction of pancreatic islet cells with recombinant adeno-associated virus vectors. *Diabetes* 50:515–520, 2001
3. Becker T, Noel RJ, Coats WS, Gomez-Foix A, Alam T, Gerard RD, Newgard CB: Use of recombinant adenovirus for metabolic engineering. In *Methods Cell Biology*. Roth M, Ed., New York, Academic Press, p. 161–189, 1994
4. Bain J, Schisler JC, Taekuchi K, Newgard CB, Becker TC: An adenovirus vector for efficient RNAi-mediated suppression genes in insulinoma cells and pancreatic islets of Langerhans. *Diabetes* 53:2190–2194, 2004
5. Ayuso E, Chillón M, Agudo J, Haurigot V, Bosch A, Carretero A, Otaegui PJ, Bosch F: In vivo gene delivery to pancreatic beta cells by systemic delivery of adenoviral vectors. *Human Gene Ther* 15:805–812, 2004
6. Rehman KK, Bertera S, Bottino R, Balamurugan AN, Mai JC, Mi Z, Trucco M, Robbins PD: Protection of islets by in situ peptide-mediated transduction of the Ikappa B kinase inhibitor nemo-binding domain peptide. *J Biol Chem* 278:9862–9868, 2003
7. Paty BW, Bonner-Weir S, Laughlin MR, McEwan AJ, Shapiro AM: Toward development of imaging modalities for islets after transplantation: insights from the National Institutes of Health workshop on beta cell imaging. *Transplantation* 77:1133–1137, 2004
8. Barry MA, Dower WJ, Johnston SA: Toward cell-targeting gene therapy vectors: selection of cell-binding peptides from random peptide-presenting phage libraries. *Nat Med* 2:299–305, 1996
9. Pasqualini R, Ruoslahti E: Organ targeting *in vivo* using phage display peptide libraries. *Nature* 380:364–366, 1996
10. Trepel M, Arap W, Pasqualini R: In vivo phage display and vascular heterogeneity: implications for targeted medicine (Review). *Curr Opin Chem Biol* 6:399–404, 2002
11. Rajotte D, Arap W, Hagerdorn M, Koivunen E, Pasqualini R, Ruoslahti E: Molecular heterogeneity of the vascular endothelium revealed by *in vivo* phage display. *J Clin Invest* 102:430–437, 1998
12. Kulkarni RN: The islet β -cell. *Int J Biochem Cell Biol* 36:365–371, 2004
13. Brown KC: New approaches for cell-specific targeting: identification of cell-selective peptides from combinatorial libraries (Review). *Curr Opin Chem Biol* 4:16–21, 2000
14. Oyama T, Sykes KF, Samli KN, Minna JD, Johnston SA, Brown KC: Isolation of lung tumor specific peptides from a random peptide library: generation of diagnostic and cell-targeting reagents. *Cancer Lett* 202:219–230, 2003
15. McGuire MJ, Samli KN, Johnston SA, Brown KC: In vitro selection of a peptide with high selectivity for cardiomyocytes *in vivo*. *J Mol Biol* 342:171–182, 2004
16. McGuire MJ, Sykes KF, Samli KN, Barry MA, Stemke-Hale KA, Tagliaferri F, Logan M, Jansa K, Takashima A, Brown KC, Johnston SA: A library selected Langerhans cell-targeting peptide enhances an immune response. *DNA Cell Biol* 23:742–752, 2004
17. Zhou X, Chang Y, Oyama T, McGuire MJ, Brown KC: Cell-specific delivery of a chemotherapeutic to lung cancer cells. *J Am Chem Soc* 129:15656–15657, 2004
18. Cwirla SE, Peters EA, Barrett RW, Dower WJ: Peptides on phage: a vast library of peptides for identifying ligands. *Proc Natl Acad Sci U S A* 87:6378–6382, 1990
19. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB: Isolation of INS-1-derived cell lines with robust ATP-sensitive K^+ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes* 49:424–430, 2000
20. Clark J, Palmer CJ, Shaw WN: The diabetic Zucker fatty rat. *Proc Soc Exp Biol Med* 173:68–75, 1975
21. Peterson RG, Shaw WN, Neel MA, Little LA: Zucker diabetic fatty as a model for non-insulin-dependent diabetes mellitus. *ILAR News* 32:16–19, 1990
22. Shibata A, Ludvigsen CW, Naber SP, McDaniel ML, Lacy PE: Standardization of a digestion-filtration method for isolation of pancreatic islets. *Diabetes* 25:667–672, 1976
23. Lee Y, Hirose H, Zhou YT, Esser V, McGarry JD, Unger RH: Increased lipogenic capacity of the islets of obese rats: a role in the pathogenesis of NIDDM. *Diabetes* 46:408–413, 1997
24. Bonnycastle LLC, Menendez A, Scott JK: General phage methods. In *Phage Display: A Laboratory Manual*. Barbas C, Burton D, Scott J, Silverman G, Eds. Cold Spring Harbor, NY, Cold Spring Harbor Press, p. 15.11–15.30, 2000
25. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nuc Acids Res* 25:3389–3402, 1997
26. Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB: Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* 130:167–178, 1992
27. Dubowchik GM, Walker MA: Receptor-mediated and enzyme-dependent targeting of cytotoxic anticancer drugs. *Pharmacol Ther* 83:67–123, 1999
28. Green MC, Murray JL, Hortobagyi GN: Monoclonal antibody therapy for solid tumors. *Cancer Treat Rev* 26:269–286, 2000
29. American Cancer Society: Monoclonal antibody therapy [article online], 2005. Available at http://www.cancer.org/docroot/ETO/content/ETO_1_4x_Monoclonal_Antibody_Therapy_Passive_Immunotherapy.asp?sitcarea=ETO. Accessed 11 May 2005
30. Aaen K, Rygaard J, Josefsen K, Petersen H, Brogren CH, Horn T, Buschard K: Dependence of antigen expression on functional state of beta-cells. *Diabetes* 39:697–701, 1990
31. Moore A, Bonner-Weir S, Weissleder R: Noninvasive *in vivo* measurement of β -cell mass in mouse model of diabetes. *Diabetes* 50:2231–2236, 2001
32. Shimabukuro M, Zhou YT, Levi M, Unger RH: Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A* 95:2498–2502, 1998
33. Finegood DT, McArthur MD, Kojwang D, Thomas MJ, Topp BG, Leonard T, Buckingham RE: β -cell mass dynamics in Zucker diabetic fatty rats: rosiglitazone prevents the rise in net cell death. *Diabetes* 50:1021–1029, 2001