Targeted Expression of Placental Lactogen in the Beta Cells of Transgenic Mice Results in Beta Cell Proliferation, Islet Mass Augmentation, and Hypoglycemia*

Received for publication, December 6, 1999, and in revised form, February 2, 2000

The factors that regulate pancreatic beta cell proliferation are not well defined. In order to explore the role of murine placental lactogen (PL)-I (mPL-I) in islet mass regulation in vivo, we developed transgenic mice in which mPL-I is targeted to the beta cell using the rat insulin II promoter. Rat insulin II-mPL-I mice displayed both fasting and postprandial hypoglycemia (71 and 105 mg/dl, respectively) as compared with normal mice (92 and 129 mg/dl; p < 0.00005 for both). Plasma insulin concentrations were inappropriately elevated, and insulin content in the pancreas was increased 2-fold. Glucose-stimulated insulin secretion by perfused islets was indistinguishable from controls at 7.5, 15, and 20 mM glucose. Beta cell proliferation rates were twice normal (p = 0.0005). This hyperplasia, together with a 20% increase in beta cell size, resulted in a 2-fold increase in islet mass (p = 0.0005) and a 1.45-fold increase in islet number (p = 0.0012). In mice, murine PL-I is a potent islet mitogen, is capable of increasing islet mass, and is associated with hypoglycemia over the long term. It can be targeted to the beta cell using standard gene targeting techniques. Potential exists for beta cell engineering using this strategy.

It is well documented that pancreatic islet mass does not remain constant during life, but adapts to changing physiologic conditions. For example, there is a marked increase in islet proliferation during pregnancy, glucose infusion, or recovery from pancreatic resection (1–7). Conversely, a reduction in islet proliferation and mass is observed in the post-partum or fasting state (1–7). Islet mass may change either as a result of proliferation, hypertrophy or apoptosis of existing islet cells, or through islet neogenesis from ductal cells (1–7). Understanding the mechanisms underlying islet growth and differentiation is essential not only from a physiologic and developmental standpoint, but is also likely to be important for the development of new treatment approaches to diabetes.

A number of factors have been implicated in the regulation of islet mass. These include glucose itself, gastrin, transforming growth factor-α, insulin-like growth factor-1, pancreatic stone peptide and the reg family of gene products, glucagon-like peptide, islet neogenesis-associated protein, parathyroid hormone-related protein (PTHrP),1 hepatocyte growth factor, growth hormone, prolactin, and the placental lactogens (PL) (1–14). Of the several factors known to affect islet growth and function in vitro, PL is one of the most potent in increasing beta cell proliferation, insulin content, and insulin secretion from islets in vitro (14, 15). The placental lactogens are members of the growth hormone/prolactin family (14, 15). In rodents, there are at least two PLs, PL-I and PL-II. PL-I is normally made in the trophoblast giant cells of the placenta, with circulating PL-I reaching peak levels during mid-gestation (14–16). Indirect evidence in vivo and in vitro has implicated PL as the primary factor responsible for the increase in islet mass and enhanced insulin secretion observed during pregnancy in rodents and presumably humans as well (14–17). To date, unique receptors for PL have not been identified. Instead, in rodents, PL interacts on the cell surface with the prolactin receptor, a member of the cytokine family of receptors, which signals through the Janus kinase pathway (14, 15, 18, 19).

Whereas the in vitro effects of PL-I on the pancreatic islet are well documented, no long term in vivo studies exploring the role of PL in islet mass regulation have been described. To directly examine the role of PL-I on islets in vivo, a transgenic mouse model was created in which the murine placental lactogen-I (mPL-I) cdNA was targeted to and expressed in beta cells under the control of the rat insulin-II promoter (RIP). These RIP-mPL-I transgenic mice were characterized for the expression of the transgene, and the long term effect of mPL-I on islet cell physiology, beta cell proliferation, and islet mass in vivo. RIP-mPL-I mice display a marked increase in islet mass, which results from both islet hyperplasia as well as hypertrophy. These changes are associated with hypoglycemia. These findings may have relevance to strategies aimed at enhancing islet mass and function in diabetes.

MATERIALS AND METHODS

Construction of the Transgene and Generation and Identification of Transgenic Mice—The RIP-mPL-I transgene consists of a 650-bp segment of the rat insulin II gene promoter (11, 20), driving transcription of the 800-bp mouse placental lactogen I (mPL-I) cDNA (21) (generously provided by Dr. Daniel Linzer, Northwestern University, Evanston, IL), downstream of which lie untranslated human growth hormone sequences providing transcriptional termination, polyadenylation, and splicing signals (11). The transgene was constructed by replacing the

1 The abbreviations used are: PTHrP, parathyroid hormone-related protein; PL, placental lactogen; mPL, murine placental lactogen; bp, base pair(s); RIP, rat insulin-II promoter; RIA, radioimmunoassay.
PThPr cDNA sequence from our previously described RIP-PThPr cDNA construct (11) with the mPL-I cDNA. The resulting 3.6-kilobase pair RIP-mPL-I transgene was isolated, purified, and microinjected into the pronuclei of (C57Bl/6 × SJL) F2 mouse ova as described earlier (11). Of the 35 founder mice obtained, 6 were transgenic as identified by polymerase chain reaction (PCR) detection of tail DNA extracted using primers and methods described previously (22). All six founders yielded true-breeding lines when bred with CD1 mice. The age group of mice used in the experiments varied from 2 to 8 months. The specific ages of mice used in particular experiments are detailed below under this section or under “Results.” For each experiment, transgenic and control mice of similar ages and gender ratios were used. No differences among age or gender groups were observed. All procedures were approved by the University of Pittsburgh Animal Care and Use Committee.

RNA Analysis—The pancreas and other tissues were harvested and immediately frozen in liquid nitrogen for subsequent RNA extraction. Total RNA from pancreas was prepared using a modification of the guanidinium thiocyanate-cesium chloride method (23), and RNA from all other tissues was made by the Trizol (Life Technologies, Inc.) method. RNase protection analysis was performed as described (11, 12), using four different cRNA probes, which protected the following sequences: (i) a 230-bp band corresponding to mouse insulin sequences protected by a PstI–AvaiI mouse insulin genomic fragment; (ii) a 220-bp band corresponding to a Sau3A–Sau3A mouse cyclophilin cDNA fragment; (iii) an approximately 230-bp band corresponding to the 3′ end of the mPL-I cDNA digested at the PstI site, and, (iv) a 115-bp band corresponding to the human 28 S RNA cDNA.

Pancractic Protein Analysis—To quantitate insulin content in the pancreas of control and transgenic mice, pancreata were dissected and immediately frozen in liquid nitrogen, and protein extracts prepared using the acid-ethanol method (24). Briefly, pancreata weighing 350–450 mg were homogenized in 5 ml of acid-ethanol, consisting of a 50:1 ratio of 95% ethanol to 10.2 N hydrochloric acid. After an overnight incubation at 4 °C, the extracts were centrifuged at 2,500 rpm for 30 min at 4 °C. Insulin RIAs were performed on the extracts as described below, and insulin concentrations were expressed as a function of pancreatic content. Pancreatic insulin content was measured in mice at 3 months of age and 8 months of age. To measure mlet mPL-I content, islets were isolated from normal and transgenic pancreas as described (25). Murine PL-I concentration in islet extracts was measured using an mPL-I RIA as described previously (16). The detection limit of the mPL-I RIA in rat insulin extract buffer is 1.0 ng/ml. Murine PL-I is expressed as a function of islet extract protein.

Plasma Analysis—Blood for glucose and insulin measurement was obtained from mice between 2 and 6 months of age by retro-orbital bleeds. Glucose was measured on whole blood using an Accu-Chek III glucometer (Roche Molecular Biochemicals). Insulin was measured by RIA as described previously (26) using a polyclonal guinea pig insulin antiserum (ICN Biomedicals, Costa Mesa, CA) and a rat insulin standard (Eli Lilly, Indianapolis, IN). For plasma mPL-I measurements, blood was obtained by cardiac puncture using mice anesthetized with methoxyflurane (Mallinckrodt Veterinary, Mundelein, IL). Normal pregnant female mice at gestational days 10–12 were used as positive controls for detection of mPL-I in plasma. Murine PL-I was measured using an mPL-I RIA with a detection limit in plasma of 4.5 ng/ml as described previously (16).

Immunohistochemistry and Quantitative Islet Histomorphometry—The entire pancreas was removed and fixed in Bouin’s solution for 12–16 h. Sections were prepared and immunostained for insulin, glucagon, pancreatic polypeptide, and somatostatin as described previously (11, 12). Immunohistochemistry for mPL-I was performed using a rabbit polyclonal antiserum (27) at a dilution of 1:1000. Sections were microwaved for 10 min in 10 mM sodium citrate buffer, pH 6.0, then incubated for 2 h at room temperature with primary antiserum, and then exposed to the biotinylated secondary antibody and avidin-biotin complex (Biogenex, San Ramon, CA). The specificity of staining was verified by incubation of the antiserum with 10–5 M mPL-I protein. Quantitative islet histomorphometry was performed in a blinded fashion, using insulin-immunostained pancreatic sections from 7 normal and 12 transgenic mice between the ages of 3 and 4 months (representing all three transgenic lines) using a Nikon Labophot microscope coupled to a computerized planimeter and analyzed using the Osteomet- sure software package (Osteometrics, Atlanta, GA) as described previously (11, 12).

Measurement of Beta Cell Replication Rate and Islet Cell Size—Beta cell proliferation was measured in 3–4-month-old transgenic and normal mice using immunohistochemical bromodeoxyuridine staining as described previously (12, 28). In order to specifically identify beta cells, pancreatic sections were co-stained with antisera to glucagon, somatostatin, and pancreatic polypeptide as described by Montana et al. (28), and only beta cells were quantitated for replication. Islet cell size was determined using hematoxylin- and eosin-stained pancreatic sections from three normal and three transgenic mice, counting at least a 1000–islet nuclei from each pancreas. Mean islet cell size was calculated by dividing the total islet area by the total number of islet nuclei (12).

Islet Perfusion—These studies were performed as described in detail previously (11). Briefly, mice between the ages of 5 and 8 months were anesthetized using Nembutal (50 mg/kg), and pancreata were removed and incubated for 5–7 min in collagenase P (5 mg/pancreas) for islet isolation (11). The digest was washed several times in Hanks’ balanced salt solution, and individual islets were manually harvested under a dissecting microscope and placed onto a nylon membrane. The membranes were then placed into perfusion chambers and perfused at a rate of 1 ml/min with glucose added in the concentrations indicated in the legend to Fig. 6. Fractions were collected at 1- or 5-min intervals as shown in Fig. 6 and insulin measured using the RIA described above. Groups of 15–20 islets were used for each perfusion assay, and care was taken to select islets of similar size.

Streptozotocin Studies—Streptozotocin (1.0 mg/50 ml in 0.9% saline containing 10 mM sodium citrate, pH 4.0) was administered intraperitoneally to normal and RIP-mPL-I mice (n = 10 each) between the ages of 2 and 6 months in two doses of 75 mg/kg separated by 12 h. Tail blood glucose concentrations were obtained at the times indicated in Fig. 9.

Statistical Analysis—Statistics were determined using Student’s two-tailed t test with unequal variance, and using one-way analysis of variance as described in the figure legends. p values less than 0.05 were considered significant.

RESULTS

Expression of the RIP-mPL-I Transgene—Expression of the transgene in the pancreas of each of the six founder lines was assessed using RNase protection analysis of total pancreatic RNA from normal and transgenic mice (Fig. 1). As expected, there was no expression of the endogenous mPL-I gene in the pancreas of normal mice. The mPL-I transgene, on the other hand, was expressed at varying levels in the pancreata of all six transgenic lines of mice. Based on the level of RNA expression, the three highest expressers, lines 48, 60, and 64, were chosen for subsequent studies. To determine whether the transgene was expressed in tissues other than the islet, RNase protection analysis was performed on total RNA from several organs of these three RIP-mPL-I lines. As seen in Fig. 2, line 60 expressed mPL-I mRNA in the liver and kidney, and to a lesser extent, in intestine and heart. In line 64, the transgene was expressed in the brain, but not other tissues. In line 48, expression was limited to the pancreas. This is particularly important, as line 48 animals display all of the features of the phenotype described below.

To determine whether transgene mRNA resulted in mPL-I protein production in the islet, immunohistochemistry using an

**Fig. 1. Levels of expression of the RIP-mPL-I transgene in the pancreas of the six transgenic founder lines.** RNase protection analysis was performed on total RNA from two pancreata each from normal (nl) and the six RIP-mPL-I transgenic lines (lines 44, 48, 55, 60, 64, and 68). Note that there is no expression of the endogenous mPL-I gene in normal pancreas, but a varying degree of expression of the mPL-I transgene in the pancreas of all six transgenic lines. Transgene expression is not apparent in the pancreas of lines 44 and 55 at this duration of exposure, but becomes clearly apparent at longer exposures. The cyclophilin probe was used as a housekeeping gene control for sample loading, and the digested probe lane represents the negative control with no RNA. Based on this information, lines 48, 60, and 64 were selected for further study.
anti-mPL-I antiserum was performed on sections of whole pancreas from normal and transgenic mice (Fig. 3). As demonstrated in the figure, mPL-I protein is made specifically in the islets of transgenic mice (Fig. 3, A and B), with no staining observed in the exocrine tissue and only faint, nonspecific staining seen in islets of normal littermates (Fig. 3C). Immunohistochemistry performed in the presence of excess mPL-I peptide markedly diminished the staining of transgenic mouse islets to a nonspecific background level (Fig. 3D), indicating that the staining in panels A and B was specific for mPL-I. The figure shown represents line 60, but similar staining was observed in lines 48 and 64. Thus, all three transgenic lines express mPL-I in the pancreatic islet as determined immunohistochemically.

Murine PL-I was quantitated using an mPL-I RIA in protein extracts of islets of transgenic and normal mice. Using batches of 120–150 islets for each extraction, line 60 was found to have an average of 235 ± 124 (mean ± S.D.) pg of mPL-I/µg of islet protein (n = 11), whereas lines 48 and 64 contained lower amounts, 19 ± 11 (n = 8) and 16 ± 0.6 (n = 10) pg of mPL-I/µg of islet protein, respectively. Extracts of normal islets contained unmeasurable amounts of mPL-I, less than 1–2 pg/µg. Thus, mPL-I is clearly measurable by RIA in extracts from islets of all three transgenic lines, with line 60 displaying values some 10-fold higher than lines 48 and 64. Since mPL-I is a secretory protein, it is likely to be released from the islets of transgenic mice. Thus, circulating concentrations of mPL-I were measured in the plasma of normal and RIP-mPL-I mice. No mPL-I was detected in the plasma of either normal or transgenic mice (n = 12 each) using an assay with a detection limit in plasma of 4.5 ng/ml. In marked contrast, plasma from normal pregnant female mice (n = 5) at gestational days 10–12, used as a positive control, contained strikingly elevated concentrations of mPL-I, ranging from 30 to 3,600 ng/ml, as reported previously (16), some 5–700 times the assay detection limit.

**RIP-mPL-I Mice Are Hypoglycemic and Inappropriately Hyperinsulinemic**—To determine whether mPL-I expression in the islet might affect glucose-insulin homeostasis in the RIP-mPL-I mouse, these parameters were measured in 2–6-month-old normal and transgenic mice under both fasting and nonfasting conditions. As seen in Fig. 4 (upper panel), blood glucose concentrations in the RIP-mPL-I mice were distinctly lower than those in normal littermates under both conditions. The mean postprandial blood glucose concentration in normal littersmates was 128.8 mg/dl, and fell to 92.1 mg/dl following a 24-h fast. In contrast, transgenic littersmates from each of the three lines had significantly lower blood glucose values, 105.2 mg/dl under post-prandial conditions, which were further reduced to 70.9 mg/dl after 24 h of fasting. When the blood glucose values were examined in each of the three individual transgenic lines, the mean fasting glucose for line 60 was 69.1 mg/dl (n = 21); for line 48, 78.6 mg/dl (n = 11); and, for line 64, 65.6 mg/dl (n = 9), as compared with 92.1 for normal littersmates (n = 29). Similarly, the non-fasting blood glucoses were lower in each of the three transgenic lines, as follows: line 60, 104.1 mg/dl (n = 31); line 48, 106.4 mg/dl (n = 14); and line 64, 106.8 (n = 11) as compared with a mean of 128.8 mg/dl for control animals (n = 46). In each case, the individual transgenic lines were statistically significantly different from the normal mean. Thus, the RIP-mPL-I mice were relatively hypoglycemic as compared with their normal littersmates under both fasting as well as non-fasting conditions, and this relative hypoglycemia was observed in all three lines. No differences were observed as a function of gender or of age among these mice in the 2–6-month age range.

Plasma insulin concentrations measured on the same samples are shown in Fig. 4 (middle panel). There was no difference in the insulin levels between normal and transgenic mice in either the fasting or postprandial state. A comparison of the insulin-to-glucose ratios for normal and RIP-mPL-I mice (Fig. 4, lower panel), suggests that insulin concentrations are inappropriately elevated in the transgenic mice as compared with their normal littersmates under fasting conditions.

**Insulin Content and Secretion**—Murine PL-I has been implicated in the increase in insulin content as well as the change in glucose-stimulated insulin secretory response of islets that occurs during pregnancy (14, 15, 17). Measurement of insulin content in whole pancreatic extracts (Fig. 5) revealed a 2-fold increase in the pancreata of transgenic as compared with normal mice. The glucose-stimulated insulin secretory response of islets isolated from normal and RIP-mPL-I mice was compared in vitro (Fig. 6). As seen in the figure, at the three different concentrations of glucose tested, there was no obvious difference in the profile of insulin secretion on a per islet basis from islets of transgenic as compared with normal mice.

**Beta Cell Replication, Islet Cell Size, and Islet Mass**—Murine PL-I has been shown to cause an increase in beta cell replica-
Blood samples obtained early in the morning after ad libitum feeding overnight. Bars indicate standard deviation. p values calculated using one-way analysis of variance were less than 0.05 for each of the categories that achieved p < 0.05 using Student’s t test.

Fig. 4. Whole blood glucose (upper panel), plasma insulin concentrations (middle panel), and insulin-glucose ratios (lower panel) in RIP-mPL-I mice and their normal littermates after 24-h fasting or non-fasting conditions. TG indicates RIP-mPL-I mice, N indicates normal littermates. N denotes the number of mice examined in each group, except that, for the postprandial glucose values, the n for normal mice was 46 and for transgenic mice was 56. Each n in Fig. 4 represents a single animal, for example n = 29 indicates that 29 different animals were bled to obtain the data shown in the corresponding bars. Fasting indicates a 24-h fast, and postprandial refers to blood samples obtained early in the morning after ad libitum feeding overnight. Bars indicate standard deviation, p values determined using Student’s t test are shown. p values calculated using one-way analysis of variance were less than 0.05 for each of the categories that achieved p < 0.05 using Student’s t test.

Fig. 5. Pancreatic insulin content in the RIP-mPL-I mice. A total of 8 normal and 10 transgenic pancreata were examined for their insulin content after acid-ethanol protein extraction. There is a greater than 2-fold increase in the insulin content of transgenic pancreas (15.3 µg of insulin/mg of protein) compared with normal mice (7.2 µg of insulin/mg of protein). Bars indicate standard deviation.

FIG. 4. Whole blood glucose (upper panel), plasma insulin concentrations (middle panel), and insulin-glucose ratios (lower panel) in RIP-mPL-I mice and their normal littermates after 24-h fasting or non-fasting conditions. TG indicates RIP-mPL-I mice, N indicates normal littermates. In vivo and in vitro expression of mPL-I in the islets of RIP-mPL-I transgenic mice directly results in accelerated beta cell proliferation, islet hyperplasia and hypertrophy, an increase in islet mass, inappropriate hyperinsulinemia with resultant hypoglycemia, and resistance to the diabetogenic effects of streptozotocin. These in vivo results support the findings of previous in vitro studies in which mPL-I was found to increase beta cell proliferation, insulin content, and insulin secretion (14, 15). In addition, these findings complement in vivo studies documenting an increase in beta cell proliferation and in islet mass in pregnancy (15, 17). Finally, these studies corroborate reports demonstrating that the mid-gestational rise in islet proliferation correlates temporally with the transient mid-gestational ap-
pearance of mPL-I (15–17). Taken together, these studies strongly suggest that PL is responsible for the second trimester increase in beta cell mass and function that occurs in pregnancy (15, 17).

The phenotype of the RIP-mPL-I mouse appears to be a direct consequence of mPL-I expression in the pancreatic islet, rather than an effect of a random integration event of the transgene, since three independent transgenic lines exhibited the same phenotype. That all three lines of transgenic mice expressed the transgene in their islets was documented using pancreatic mRNA analysis, immunohistochemistry, and immunoblot of extracts of isolated islets. One of the three lines, line 60, expressed the transgene at approximately 10-fold higher levels than the other two transgenic lines, but it is important that all three lines were demonstrated to express both the protein and the mRNA, and that the phenotype, including relative hypoglycemia, an increase in islet mass, and accelerated beta cell proliferation, was observed in each of the three lines.

A tissue survey demonstrated that the RIP-II promoter used herein led to low but detectable expression of the transgene in sites other than the pancreatic beta cell. These included the liver, kidney, heart, and intestine in line 60, the brain in line 64, and no other site in line 48. These findings are typical of findings reported by others (29, 30), using less sensitive techniques such as Northern blotting and immunohistochemistry, as well as RNase protection analysis as used herein. From a pathophysiologic standpoint, four important points should be made here. First, line 48 expressed the transgene only in the beta cell and yet displayed the full spectrum of the phenotype, so it is reasonable to conclude that the phenotype is a direct result of mPL-1 expression in the islet, and not of “leaky” expression in other organs. Second, in line 64, leaky expression was observed only in the brain, supporting the conclusions described in the preceding sentence. Third, it is difficult to construct a pathophysiologic scenario for line 60 in which expression of the transgene, not in the islet but in the liver, kidney, intestine, and heart could produce hypoglycemia, an increase in islet mass and proliferation, and in pancreatic insulin content. Fourth, it is important to note that in the organs and transgenic lines in which leaky expression was observed, the levels of expression in sites outside the pancreas were dramatically lower than in the pancreas. As islets comprise only 2–3% of pancreatic mass, the level of expression of mPL-1 mRNA in the pancreatic islet of lines 60 and 64 is likely to be some 50–100 times higher than in the other tissues. These findings collectively make the critical point that mPL-1 expression in the islet, and not in other tissues, is responsible for the phenotype.

Systemic mPL-I was not detected in the circulation of the RIP-mPL-I mice. This suggests that the islet phenotype was the result of local mPL-I actions within the islet. Moreover, it suggests that systemic consequences of mPL-1, such as insulin resistance, should not occur in the RIP-mPL-I mouse. The reason for the lack of measurable systemic mPL-I is not clear, but most likely reflects secretion at low levels, and/or effective clearance from the portal circulation by the liver.

Histomorphometric analysis of islet mass and number in the RIP-mPL-I mouse lines demonstrated a 2-fold increase in total islet mass, with a lesser (1.45-fold) but significant increase in
Islet number. Importantly, the increase in islet mass and number was observed in each of the three transgenic lines. Whether the increase in islet number is real or is a histomorphometric reflection of an increase in islet size remains to be determined.

Fig. 8. Quantitative islet histomorphometric analysis of normal (NL) and RIP-mPL-I pancreas. Islet volume as a function of total pancreatic volume (panel A) and islet number expressed as a function of total pancreatic area (panel B) are higher in transgenic (L60, L64/48) than in normal littermates. Each symbol represents an individual animal, and the lines indicate the means.

accompanied by inappropriately normal insulin concentrations in plasma. Thus, it may be possible that a minor increase in islet mass could, independent of individual beta cell function, lead to hyperinsulinemia. Alternatively, or in addition, hyperinsulinemia could reflect the abnormal functioning of individual islet cells, resulting in increased insulin synthesis, and/or a lowered glucose-stimulated insulin secretion threshold with resultant increases in insulin secretion. Placental lactogen has been shown to have all of the above effects on islets or on the isolated perfused pancreas in vitro (14, 15, 17). We presume that the inappropriate hyperinsulinemia in the RIP-mPL-I mouse is attributable to a combination of the above factors. Finally, it is formally possible that placental lactogen secreted into the portal circulation may contribute to the hypoglycemia observed, perhaps by altering hepatic glucose uptake or glucose production. This cannot be the primary pathophysiological feature of the phenotype, as it would not explain the increase in insulin production and the increase in islet mass and proliferation. These considerations support the concept that the primary action of placental lactogen is a direct effect on the beta cell to increase its mass and function.

One might postulate that the hypoglycemia in the RIP-mPL-I mouse could be due to increased periperal insulin sensitivity, since hypoglycemia is present in the presence of normal insulin concentrations. At least two considerations make this hypothesis unlikely. First, there is no plausible reason for there to be increased insulin sensitivity. If anything, placental lactogen is believed to be associated with peripheral insulin resistance. Second, if this mechanism were operative, one would still expect a reduction in circulating insulin concentrations and in islet mass and pancreatic insulin content, as occurs, for example in the non-involved islets in patients and rats with insulinomas, or as occurs during prolonged fasting. These are not a feature of the RIP-mPL-I mouse.

Previous reports describe a leftward shift in the insulin secretory response curve in the pregnant pancreas (14, 15, 17). One might therefore have predicted that glucose-stimulated insulin secretion might be enhanced in isolated perfused RIP-mPL-I islets. On the other hand, one might have imagined that proliferation induced by mPL-I would be accompanied by dedifferentiation of beta cells and an associated decline in insulin secretory responses to glucose. In fact, when the insulin secretory response of isolated RIP-mPL-I islets was examined in vitro, there was no obvious difference between the insulin secretion profile of islets from transgenic mice compared with their normal littermates at the three glucose concentrations tested. Whether glucose-stimulated insulin secretion in vitro reflects the functioning of mPL-I islets in vivo is uncertain. Most previous studies examining the influence of PL or PRL on insulin secretion in vitro have employed the perfused rat pancreas (15). The few studies performed on isolated islets indicate that a minimum concentration of mPL-I of 50–60 ng/ml is required, over a period of 4 days, to observe any effects of mPL-I on glucose-stimulated insulin secretion in vitro (14, 15). It seems possible, therefore, that the mPL-I concentration in the RIP-mPL-I islets is too low to cause a change in their insulin secretion profile in vitro. It is also possible that the lack of change in glucose-stimulated insulin secretion reflects a balance of two opposing effects: de-differentiation induced by mPL-I on the one hand, neutralized or balanced by a leftward shift in the insulin response to glucose on the other hand. Additionally, it is possible that the islet isolation process injures or alters the insulin secretory response to glucose. Based on what is known to occur in pregnancy and in vitro with mPL-1, we would anticipate that more extensive studies using larger numbers of islets, a broader range of glucose concentra-
tions, a different array of insulin secretagogues, and/or in vivo studies with the perfused mouse pancreas or in vivo glucose clamp studies in the RIP-mPL-I mouse may very well demonstrate differences in the dynamics of glucose-stimulated insulin secretion by RIP-mPL-I mouse islets as compared with normal islets.

The primary rationale for preparing the RIP-mPL-I mouse was to explore the potential therapeutic role for PL in enhancing islet mass and/or function in diabetes. With this idea in mind, streptozotocin was employed to induce diabetes. The RIP-mPL-I mouse was found to be streptozotocin-resistant, whereas their normal littermates exposed to the same dose of streptozotocin developed frank diabetes. The underlying physiology resulting in the maintenance of euglycemia remains to be defined. It may simply reflect the increase in islet mass in the RIP-mPL-I mouse, or may reflect proliferative or other functional changes in the RIP-mPL-I islets. Further studies will be required to define the physiology underlying this phenomenon.

The RIP-mPL-I mouse, the RIP-HGF mouse, and the RIP-PTHrP mouse represent three in vivo transgenic mouse models of increased islet mass leading to inappropriate hyperinsulinemia and hypoglycemia. The three islet-targeted proteins, mPL-I, HGF, and PTHrP, act via different receptors coupled to different intracellular signaling pathways (protein kinases A and C in the case of PTHrP (32), MAP kinase in the case of HGF (31), and Janus kinase in the case of mPL-I (18, 19)). Whereas they result in what appear to be superficially similar phenotypes, the mechanisms by which PTHrP, HGF, and mPL-I increase islet mass in their respective transgenic mouse models are quite different; mPL-I and HGF cause accelerated beta cell proliferation (with a component of islet cell hypertrophy as well in the case of mPL-I), whereas PTHrP seems to affect neither, and instead likely acts by slowing the normal rate of beta cell turnover (11, 12). These differences in signaling pathways and phenotypes may suggest that delivery of two or more of these proteins simultaneously to the beta cell might have synergistic effects. Such studies are under way.

Finally, at one level, the targeted delivery of mPL-I to the beta cell may seem pedestrian in the sense that the phenotype was largely predictable from prior in vitro studies. On the other hand, the finding that tissue-specific delivery to the beta cell of factors such as PTHrP, HGF, and mPL-I, which enhance islet mass and function, has clear implications for strategies aimed at enhancing islet mass and function in patients with diabetes.

Acknowledgments—We thank Dr. Daniel Linzer for providing the mPL-I cDNA. We also thank Kathy Zawalich, Lisa Nguyen, Darinka Sipula, and Dr. Vasu Reddy for technical assistance. We thank Dr. Simon Watkins and Ciprian Almonte for help with imaging.

REFERENCES
1. Finegood, D. T., Scaglia, L., and Bonner-Weir, S. (1995) Diabetes 44, 249–56
2. Swenne, I. (1992) Diabetologia 35, 193–201
3. Kore, M. (1993) J. Clin. Invest. 92, 1113–1114
4. Efrat, S. (1996) Diabetes Rev. 4, 224–234
5. Bonner-Weir, S., Deery, D., Leahy, J. L., and Weir, G. C. (1989) Diabetes 38, 49–53
6. Miyaura, C., Chen, L., Appel, M., Alam, T., Inman, L., Hughes, S. D., Milburn, J. L., Unger, R. H., and Newgard, C. B. (1991) Mol. Endocrinol. 5, 228–234
7. Scaglia, L., Smith, F. E., and Bonner-Weir, S. (1995) Endocrinology 136, 5461–5468
8. Swenne, I. (1985) Diabetes 34, 803–807
9. Wang, T. C., Bonner-Weir, S., Oates, P. S., Chulak, M. B., Simon, B., Merlino, G. T., Schmidt, E. V., and Brand, S. J. (1993) J. Clin. Invest. 92, 1349–1356
10. Rafaeloff, R., Pittenger, G. L., Barlow, S. W., Qin, X. F., Yan, B., Rosenberg, L., Duguid, W. F., and Vinik, A. I. (1997) J. Clin. Invest. 99, 2105–2109
11. Vasavada, R. C., Cavaliere, C., D’Ercole, A. J., Dann, P., Burton, W. J., Madlener, A. L., Zawalich, K., Zawalich, W., Philbrick, W. M., and Stewart, A. F. (1996) J. Biol. Chem. 271, 1200–1208
12. Porter, S. E., Sorenson, R. L., Dann, P., Garcia-Ocaña, A., Stewart, A. F., and Vasavada, R. C. (1998) Endocrinology 139, 3743–3751
13. Otonkoski, T., Cirulli, V., Beattie, G. M., Soto, G., Rubin, J. S., Hayek, A. (1996) Endocrinology 137, 3131–3139
14. Breije, T. C., Scharp, D. W., Lacy, P. E., Ogren, L., Talamantes, F., Robertson, M., Friesen, H. G., and Sorenson, R. L. (1996) Endocrinology 131, 879–887
15. Breije, T. C., Sorenson, R. L. (1997) J. Clin. Invest. 99, 301–307
16. Ogren, L., Southard, J. N., Colosi, P., Linzer, D. I. H., and Talamantes, F. (1989) Endocrinology 125, 2253–2257
17. Parsons, J. A., Breije, T. C., and Sorenson, R. L. (1992) Endocrinology 130, 1459–1466
18. Bole-Feyso, C., Goffin, V., Edery, M., Binart, N., and Kelley, P. A. (1998) Endocr. Rev. 19, 225–268
19. Stout, L. E., Sorenson, A. M., and Sorenson, R. L. (1997) Endocrinology 138, 1592–1605
20. Hanahan, D. (1985) Nature 315, 115–122
21. Colosi, P., Talamantes, F., and Linzer, D. I. H. (1987) Mol. Endocrinol. 1, 767–775
22. Weir, E. C., Philbrick, W. M., Amling, M., Neff, L. A., Baron, R., and Broadsus, A. E. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10240–10245
23. Chiangwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
24. Davalli, A. M., Ogawa, Y., Scaglia, L., Wu, Y. J., Hallister, J., Bonner-Weir, S., and Weir, G. C. (1995) Diabetes 44, 104–111
25. Gotob, M., Maki, T., Kiyoiizumi, T., Satomi, S., and Menaow, A. P. (1985) Transplantation 40, 437–438
26. Zawalich, W. S., Zawalich, K. C., Ganesan, S., Calle, R., and Rasmussen, H. (1991) Biochem. J. 278, 49–56

FIG. 9. Effects of streptozotocin administration on blood glucose concentrations in RIP-mPL-I mice (squares) (n = 10) and normal littermates (triangles) (n = 10). Note that the RIP-mPL-I mice begin mildly hypoglycemic as compared with their normal littermates and remain euglycemic following streptozotocin treatment, whereas their normal littermates progress to frank diabetes mellitus. Bars indicate standard deviation.
27. Colosi, P., Ogren, L., Southard, J. N., Thordarson, G., Linzer, D. I., and Talamantes, F. (1988) Endocrinology 123, 2662–2667
28. Montana, E., Bonner-Weir, S., and Weir, G. C. (1994) J. Clin. Invest. 93, 1577–1582
29. Lo, D., Burkly, L. C., Widera, G., Cowing, C., Flavell, R. A., Palmiter, R. D., and Brinster, R. L. (1988) Cell 53, 159–168
30. Picarella, D. E., Kratz, A., Li, C.-B., Ruddle, N. H., and Flavell, R. A. (1992) Proc. Nat. Acad. Sci. U. S. A. 89, 10036–10040
31. Garcia-Ocaña, A., Takane, K., Syed, M. A., Phillbrick, W. M., Vasavada, R. C., and Stewart, A. F. (2000) J. Biol. Chem. 275, 1226–1232
32. Vasavada, R. C., Garcia-Ocaña, A., Massfelder, T., Dann, P., and Stewart, A. F. (1998) Rec. Prog. Hormone Res. 53, 305–340
Targeted Expression of Placental Lactogen in the Beta Cells of Transgenic Mice Results in Beta Cell Proliferation, Islet Mass Augmentation, and Hypoglycemia
Rupangi C. Vasavada, Adolfo Garcia-Ócaña, Walter S. Zawalich, Robert L. Sorenson, Pamela Dann, Mushtaq Syed, Linda Ogren, Frank Talamantes and Andrew F. Stewart

J. Biol. Chem. 2000, 275:15399-15406.
doi: 10.1074/jbc.275.20.15399

Access the most updated version of this article at http://www.jbc.org/content/275/20/15399

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 9 of which can be accessed free at http://www.jbc.org/content/275/20/15399.full.html#ref-list-1