Islet cell proliferation and apoptosis in insulin-like growth factor binding protein-1 in transgenic mice

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Abstract

Transgenic mice which overexpress insulin-like growth factor binding protein-1 (IGFBP-1) demonstrate fasting hyperglycemia, hyperinsulinemia and glucose intolerance in adult life. Here we have examined the ontogeny of pancreatic endocrine dysfunction and investigated islet cell proliferation and apoptosis in this mouse model. In addition we have examined pancreatic insulin content in transgenic mice derived from blastocyst transfer into non-transgenic mice. Transgenic mice were normoglycemic at birth but had markedly elevated plasma insulin levels, 56·2 ± 4·5 versus 25·4 ± 1·5 pmol/l, *P*<0·001, and pancreatic insulin concentration, 60·5 ± 2·5 versus 49·0 ± 2·6 ng/mg of tissue, *P*<0·01, compared with wild-type mice. Transgenic mice derived from blastocyst transfer to wild-type foster mothers had an elevated pancreatic insulin content similar to that seen in pups from transgenic mice. There was an age-related decline in pancreatic insulin content and plasma insulin levels and an increase in fasting blood glucose concentrations, such that adult transgenic mice had significantly less pancreatic insulin than wild-type mice. Pancreatic islet number and the size of mature islets were increased in transgenic animals at birth compared with wild-type mice. Both islet cell proliferation, measured by 5-bromo-2'-deoxyuridine labeling, and apoptosis, assessed by the in situ terminal deoxynucleotidyl transferase and nick translation assay, were increased in islets of newborn transgenic mice compared with wild-type mice. In adult mice both islet cell proliferation and apoptosis were low and similar in transgenic and wild-type mice. Islets remained significantly larger and more numerous in adult transgenic mice despite a reduction in pancreatic insulin content. These data suggest that overexpression of IGFBP-1, either directly or indirectly via local or systemic mechanisms, has a positive trophic effect on islet development.

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Introduction

The insulin-like growth factors (IGFs) are present in the circulation and the extracellular space bound to high affinity insulin-like growth factor binding proteins (IGFBPs). Of the six IGFBPs so far characterized, IGFBP-1 appears to be the most important in regulating the insulin-like activity of the IGFs in response to acute changes in nutritional state (Lee et al. 1993). Plasma IGFBP-1 concentrations are inversely regulated by insulin (Suikkari et al. 1989, Murphy et al. 1991). Insulin suppression of hepatic IGFBP-1 transcription, mediated via the insulin response element in the 5' flanking region of the IGFBP-1 gene, has been studied in detail by a number of laboratories (Unterman et al. 1991, Suwanickul et al. 1993, Robertson et al. 1994).

In rats, IGFBP-1 infusion causes mild hyperglycemia suggesting that the small amount of free IGF-I in the circulation has a significant hypoglycemic effect (Lewitt et al. 1991). Our recent report of fasting hyperglycemia, fasting hyperinsulinemia and impaired glucose tolerance in adult transgenic mice which constitutively overexpress IGFBP-1 (Rajkumar et al. 1995, 1996a) supports this earlier observation and indicates that the interaction between free IGF-I and IGFBP-1 may be important in regulating glucose homeostasis. The hyperinsulinemia and hyperglycemia observed in adult IGFBP-1 transgenic mice can not be attributable to insulin resistance alone since there is no demonstrable resistance to the hypoglycemic effect of subcutaneous insulin or resistance to the effect of insulin on glucose uptake by isolated adipocytes (Rajkumar et al. 1996a,b) and only modest skeletal muscle insulin resistance in adult transgenic mice (Rajkumar et al. 1996b).

We have previously observed that adult transgenic mice have an increase in the relative weight of the pancreas and larger and more numerous pancreatic islets (Rajkumar et al. 1996a). In the present study we have examined the ontogeny of pancreatic endocrine dysfunction and the age-related changes in islet cell proliferation and apoptosis in the transgenic mouse model. In addition we have used blastocyst transfer to assess the potential contribution of maternal factors in transgenic mice to pancreatic function.
Materials and Methods

Transgenic mice

Transgenic mice overexpressing IGFBP-1 were generated using rat genomic fragment, containing the entire coding region of the IGFBP-1 gene including the 3’ untranslated sequence, the 5’ untranslated region and 78 bp of 5’ flanking DNA, inserted downstream of the mouse phosphoglycerate kinase promoter (Rajkumar et al. 1995). Homozygous transgenic mice of the 277A strain were used for all experiments. Wild-type mice of the same genetic background were generated from non-transgenic offspring of founder mice and bred in a similar fashion to the transgenic mice. All animal experimentation was performed in accordance with protocols approved by the Animal Care Committee of the Faculty of Medicine, University of Manitoba.

Blood glucose determination

Glucose was measured in whole blood from overnight fasted mice using a YSI 2300 glucose analyzer (Yellow Springs, OH, USA).

Insulin extraction and immunoassay

Pancreatic insulin was extracted as previously described (Pickleers et al. 1985). The insulin concentrations in fasting plasma samples and pancreatic extracts were measured by RIA (Pharmacia Canada Inc., Baie D’Urfé, PQ, Canada). This assay recognizes mouse and human insulin equally. Human proinsulin and c-peptide show 41% and 0·18% cross-reaction respectively.

Pancreatic morphometric and immunohistochemical analysis

The mice were killed with an overdose of Avertin and the whole pancreas was carefully dissected out and fixed in 10% buffered formalin. Pancreatic tissue from between four and seven mice was examined for each time point. The tissue was embedded in paraffin and 6 µm serial sections were cut and stained with hematoxylin and eosin. Sections were examined by light microscopy. The islets were counted in every fifteenth section from the entire pancreas and expressed as the mean ± s.e.m. number of islets per mm². Between 15 and 20 mature islets from each mouse were chosen randomly from various regions of the pancreas for determination of islet size, using an ocular micrometer. For immunohistochemical studies, 1-day-old and adult Tg and Wt mice were killed with an overdose of Avertin and the whole pancreas was carefully dissected out and fixed in 4% paraformaldehyde in 0·1 M phosphate buffer, pH 7·4 for 5 h at 4 °C and stored overnight in phosphate buffer containing 20% sucrose at 4 °C. Frozen sections, 8 µm thick, were cut and mounted on gelatinized slides. Glucagon-, insulin-, somatostatin- and pancreatic polypeptide-containing cells were localized in adjacent serial sections using the avidin–biotin peroxidase complex method. Sections were initially blocked with 4% normal goat serum (NGS) in phosphate buffer and subsequently incubated for 24 h at 4 °C with either guinea pig anti-insulin antiserum (1:500 diluted in NGS), rabbit anti-glucagon (1:1000), rabbit anti-somatostatin (1:500) antiserum or guinea pig anti-pancreatic polypeptide antiserum (1:500). The anti-pancreatic polypeptide antiserum was obtained from Linco Research Inc. (St Louis, MO, USA) whereas the other antisera were purchased from Incstar Corporation (Stillwater, MN, USA). The antigen–antibody complex was visualized using biotinylated second antibody and avidin–peroxidase (Vector Laboratories Inc., Burlingame, CA, USA). Sections were examined by light microscopy. The actual number of positive cells with each of the antibodies was determined in 10 to 15 islets from 3 Tg and Wt mice.

Bromodeoxyuridine labeling studies

Four neonatal, 1–day-old, and four adult mice received an i.p. injection of 5-bromo-2’-deoxyuridine (BrdU, Sigma Chemical Co.), 120 µg/g body weight, dissolved in 7 mM NaOH 150 mM NaCl and were killed 16 h later. Pancreatic tissue was dissected out and immersed in 4% paraformaldehyde in 0·1 M phosphate buffer containing 2·8% sucrose at 4 °C and was subsequently stored in phosphate buffer containing 20% sucrose buffer at 4 °C overnight. Frozen sections of 8 µm were cut. Sections were pretreated with 2·8 M HCl for 15 min and blocked with 4% normal rabbit serum (NRS) for 1 h at room temperature. Sections were then incubated for 24 h at 4 °C with a 1:100 dilution of rat anti-BrdU monoclonal antibody (Sera Lab Ltd, Sussex, UK) in 1% NRS and subsequently with biotinylated anti-rat IgG, mouse adsorbed and raised in rabbit (Vector Laboratories Inc.) for 1 h at room temperature. The sections were treated with 1% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase. Antigen–antibody complexes were detected using avidin–peroxidase with 2·8-diaminobenzidine (DAB) as substrate. Sections were counterstained with hematoxylin. A set of pancreatic sections, collected from a new–born pup which had not been injected with BrdU, was processed with experimental sections as negative control.

Apoptosis

Programmed cell death was detected in pancreatic islets using the method of Gavrieli et al. (1992). The pancreatic tissues from three 1–day-old mice and three adult mice were fixed in 4% paraformaldehyde in 0·1 M phosphate buffer (pH 7·4). Frozen sections, 8 µm thick, were cut and incubated with 2 µg/ml proteinase K for 15 min at room temperature. Serial serial sections using the avidin–biotin peroxidase complex method. Sections were initially blocked with 4% normal goat serum (NGS) in phosphate buffer and subsequently incubated for 24 h at 4 °C with either guinea pig anti-insulin antiserum (1:500 diluted in NGS), rabbit anti-glucagon (1:1000), rabbit anti-somatostatin (1:500) antiserum or guinea pig anti-pancreatic polypeptide antiserum (1:500). The anti-pancreatic polypeptide antiserum was obtained from Linco Research Inc. (St Louis, MO, USA) whereas the other antisera were purchased from Incstar Corporation (Stillwater, MN, USA). The antigen–antibody complex was visualized using biotinylated second antibody and avidin–peroxidase (Vector Laboratories Inc., Burlingame, CA, USA). Sections were examined by light microscopy. The actual number of positive cells with each of the antibodies was determined in 10 to 15 islets from 3 Tg and Wt mice.

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temperature. The sections were washed four times with PBS, treated with 2% hydrogen peroxide for 5 min and immersed in 30 mM Tris buffer, pH 7.2, 140 mM sodium cacodylate, 1 mM cobalt chloride. The sections were incubated with 5 U TdT and biotin-16-dUTP (final concentration 0.01 nmol/µl; Boehringer-Mannheim, Dorval, PQ, Canada) for 1 h in a humid atmosphere at 37°C. The reaction was terminated by dilution with a 300 mM NaCl, 30 mM sodium citrate solution. The sections were rinsed with PBS and covered with 2% BSA for 10 min at room temperature. The sections were then incubated with streptavidin-peroxidase (1:1000) for 30 min at 37°C and stained with DAB.

In each experiment, after proteinase K treatment, the control sections were treated with DNase (500 ng/ml) dissolved in 30 mM Tris, pH 7-2, 140 mM potassium cacodylate, 4 mM MgCl2, 0.1 mM dithiothreitol for 10 min at room temperature. These positive control sections were then washed extensively in PBS and processed with experimental sections. As negative control, a set of sections was processed without the TdT enzyme.

Blastocyst harvest and transfer

Blastocyst transfer into wild-type foster mothers was used to determine whether the maternal hyperglycemia environment present in transgenic mice had any effect on islet development in the fetus. Blastocysts were harvested by uterine flushing 3-5 days after a vaginal plug was observed. An equal mixture of transgenic and wild-type blastocysts were transferred using standard techniques (Hogan et al. 1986), to transgenic and non-transgenic foster mothers which had been mated with vasectomized mice 2-5 days prior to transfer. Southern blot analysis of tail DNA with a transgene specific probe (Rajkumar et al. 1995) was used to identify the transgenic pups. Pancreatic insulin content was determined on day 1 of life in wild-type and transgenic pups from the same blastocyst transfer and litter.

Statistical analysis

Data are presented as the mean ± S.E.M. Significant differences between groups were determined for various parameters using Student’s t-test.

Results

The age-related changes in the blood glucose, insulin and the insulin to glucose ratio for transgenic mice are shown in Table 1. Blood glucose levels in transgenic mice were not significantly different from wild-type mice at birth but rapidly rose thereafter and were significantly elevated in transgenic mice from the second week of life. In transgenic mice, the insulin levels, and the insulin to glucose ratio were significantly elevated at birth and declined towards normal as the blood glucose rose.

An age-related change in pancreatic insulin content was also observed (Table 1). Pancreatic insulin content rose in the first week after birth in both transgenic and wild-type mice and declined thereafter in both groups of mice. In newborn transgenic pups, pancreatic insulin levels were significantly higher than wild-type mice, whereas, after weaning, the pancreatic insulin content was significantly lower in transgenic mice than wild-type mice.

Since mature transgenic mice are hyperglycemic and demonstrate impaired glucose tolerance (Rajkumar et al.

<table>
<thead>
<tr>
<th>Age</th>
<th>Glucose (mmol/l)</th>
<th>Insulin (pmol/l)</th>
<th>Insulin/glucose ratio</th>
<th>Pancreatic insulin (ng/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Wild-type</td>
<td>2.91 ± 0.14</td>
<td>25.4 ± 1.54</td>
<td>8.72 ± 0.68</td>
</tr>
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<td></td>
<td>Transgenic</td>
<td>3.23 ± 0.08</td>
<td>56.2 ± 4.84 (^b)</td>
<td>17.40 ± 1.03 (^b)</td>
</tr>
<tr>
<td>Day 7</td>
<td>Wild-type</td>
<td>3.20 ± 0.12</td>
<td>21.9 ± 1.28</td>
<td>6.87 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>3.35 ± 0.09</td>
<td>52.2 ± 12.30 (^a)</td>
<td>15.60 ± 2.61 (^b)</td>
</tr>
<tr>
<td>Day 15</td>
<td>Wild-type</td>
<td>3.65 ± 0.17</td>
<td>27.6 ± 0.96</td>
<td>7.57 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>4.44 ± 0.15 (^b)</td>
<td>40.1 ± 4.50 (^b)</td>
<td>9.05 ± 0.75 (^b)</td>
</tr>
<tr>
<td>Day 30</td>
<td>Wild-type</td>
<td>2.27 ± 0.18</td>
<td>13.8 ± 0.45</td>
<td>6.07 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>4.20 ± 0.21 (^c)</td>
<td>21.4 ± 3.27 (^c)</td>
<td>5.10 ± 0.58</td>
</tr>
<tr>
<td>Day 60</td>
<td>Wild-type</td>
<td>3.40 ± 0.19</td>
<td>14.0 ± 1.09</td>
<td>4.13 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>5.88 ± 0.36 (^a)</td>
<td>27.2 ± 5.70 (^c)</td>
<td>4.62 ± 0.71</td>
</tr>
</tbody>
</table>

Data represent the mean ± s.e.m. for 6–13 mice per group. The significant differences between transgenic and wild-type mice are marked with subscripts; \(^a\) and \(^b\) indicate \(P<0.05\), \(P<0.01\) and \(P<0.001\).
a), we considered the possibility that the increased pancreatic insulin content in transgenic pups was a consequence of maternal hyperglycemia. To address this question blastocysts from homozygous transgenic mice and wild-type mice were compared. The transgenic and wild-type mice were derived from blastocysts transferred into either transgenic or wild-type foster mothers. The data represent the mean ± S.E.M. for four to six mice per group. Significant differences between the various groups are indicated.

Figure 1 The effect of maternal environment on pancreatic insulin content. The pancreatic insulin contents of 1-day-old transgenic and wild-type mice were compared. The transgenic and wild-type mice were derived from blastocysts transferred into either transgenic or wild-type foster mothers. The data represent the mean ± S.E.M. for four to six mice per group. Significant differences between the various groups are indicated.

1996a), we considered the possibility that the increased pancreatic insulin content in transgenic pups was a consequence of maternal hyperglycemia. To address this question blastocysts from homozygous transgenic mice and wild-type mice were mixed and transferred to transgenic or wild-type foster mothers. The offspring were then classified as transgenic or wild-type mice by Southern blot analysis of tail DNA. The pancreatic insulin content of non-transgenic and transgenic pups derived from the same blastocyst transfers and litters are shown in Fig. 1. Transgenic pups derived from blastocyst transfers to wild-type foster mothers had similar pancreatic insulin levels to those seen in pups derived from naturally mated transgenic mice (Fig. 1 and Table 1). Interestingly, wild-type pups derived from transgenic foster mothers did not have significantly increased pancreatic insulin content compared with wild-type pups derived from natural mating or wild-type pups transferred to wild-type foster mothers.

We have previously demonstrated an increase in islet size and number in adult transgenic mice compared with wild-type mice (Rajkumar et al. 1996a). To examine the ontogeny of these changes, islet size and number were documented at various ages (Fig. 2). Islet size increased with age in both transgenic and wild-type mice. However, islets were significantly larger in transgenic mice than wild-type mice at all ages including in the newborn period. No insulin staining of ductal cells was apparent in sections from either wild-type or transgenic mice. The number of islets in matched areas of the pancreas was determined for pancreatic tissue from transgenic and wild-type mice. A decline with age was observed in both groups of mice due to the maturation of the exocrine pancreas. However, at each time point, there were significantly more islets per mm² in pancreatic tissue in transgenic mice compared with wild-type mice (Fig. 2).

The relative proportions of the various endocrine cell types in the islets of transgenic and wild-type mice are shown in Table 2. Transgenic mouse islets contained relatively more β-cells and relatively less α-cells than islets from wild-type mice. The relative abundance of the other endocrine cells was similar in transgenic and wild-type islets.

BrdU labeling was used to quantify islet cell proliferation in transgenic and wild-type mice in newborn and adult mice. Significantly more BrdU positive cells were observed in islets from 1-day-old transgenic mice compared with wild-type mice of the same age (Fig. 3). Only a few acinar cells were labeled with BrdU and there was no significant difference between the exocrine pancreatic tissue from transgenic versus wild-type mice. No staining was seen in newborn mice which had not been injected with BrdU. When similar studies were performed in older mice very few BrdU positive cells were observed in islets from either transgenic or wild-type mice (data not shown). Since the number of BrdU positive cells was so small in islets of older mice, it was not possible to determine whether there was any significant difference between the two groups of mice.

Apoptosis was observed in islet cells from both newborn transgenic and wild-type mice. However, significantly more cells were labeled by terminal transferase in islets from transgenic mice (Fig. 4). Exocrine pancreatic tissue contained very few labeled cells using this technique and there was no apparent difference between transgenic and wild-type mice. In older animals apoptosis was less prominent and there was no apparent difference between transgenic and wild-type mice.
In previous studies we have demonstrated fasting hyperglycemia and glucose intolerance in mature transgenic mice overexpressing IGFBP-1. In these mice, insulin sensitivity as determined by the hypoglycemic response to subcutaneous insulin and insulin-stimulated glucose transport in isolated adipocytes, was normal and only a minimal reduction in insulin stimulated glucose transport was observed in muscle from the IGFBP-1 transgenic mice (Rajkumar et al. 1996a, b). Thus, in this model an important component of the hyperglycemia and impaired glucose tolerance which develops in adult life appears to be pancreatic islet cell dysfunction. In the neonatal period, IGFBP-1 transgenic mice maintained normoglycemia by increased islet cell insulin synthesis and release. The hyperinsulinemia may be due to both a compensation for the removal of the tonic hypoglycemic effect of free IGF and/or a result of insulin resistance in certain tissues. In the rodent, expression of IGF-II is rapidly downregulated in the neonatal period and the predominant IGF in the post-natal period is IGF-I (Moses et al. 1980). Early in fetal and post-natal life, normoglycemia is achieved by an increased islet cell proliferation and pancreatic insulin synthesis, storage and secretion but with increasing age there is a gradual decline in pancreatic insulin content and plasma insulin levels, and a rise in blood glucose levels. This most probably reflects a decline in insulin synthesis although this parameter was not directly measured. The time dependent decline in the ability of the islets to compensate for hyperglycemia is reminiscent of the pancreatic exhaustion which occurs in insulin-resistant pre-diabetic individuals which eventually results in frank diabetes (Porte 1991, DeFronzo et al. 1992). However, unlike other models of type II diabetes, IGFBP-1 transgenic mice are not markedly insulin resistant (Rajkumar et al. 1996b).

We have previously observed that islets from IGFBP-1 transgenic mice, when cultured in vitro, release normal or increased amounts of insulin (Dheen et al. 1996). Thus, expression of the transgene in islet cells has no significant inhibitory effect on glucose-mediated insulin release.

Data represent the mean ± S.E.M. for 9 to 15 islets from three adult mice in each group.

Superscripts a and b indicate P<0.001 and P<0.005 for the difference between wild-type and transgenic mice.

Table 2 Relative percentage of endocrine cells in islets from transgenic and wild-type mice

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Wild-type</th>
<th>Transgenic</th>
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<tbody>
<tr>
<td>β-cells</td>
<td>75.7 ± 0.4</td>
<td>79.2 ± 0.6a</td>
</tr>
<tr>
<td>α-cells</td>
<td>11.6 ± 0.5</td>
<td>9.0 ± 0.4a</td>
</tr>
<tr>
<td>D-cells</td>
<td>7.1 ± 0.6</td>
<td>7.7 ± 0.3</td>
</tr>
<tr>
<td>PP-cells</td>
<td>6.3 ± 0.4</td>
<td>6.5 ± 0.2</td>
</tr>
</tbody>
</table>

Figure 2 Age-related changes in pancreatic islet size and number in transgenic and wild-type mice. Islets were counted in matched sections from the entire pancreas and the number expressed per mm² of pancreatic tissue. The data represent the mean ± S.E.M. for three to seven mice per time point. Significant differences between the two groups are indicated as * P<0.05 and ** P<0.01.
While expression of the transgene in the islets may not affect glucose-mediated insulin release, it may affect the mitogenic activities of locally expressed and blood-derived IGFs.

Hyperinsulinemia and increased islet size and number were apparent in newborn transgenic mice indicating that the mechanisms underlying these changes are operative prior to birth. Hyperglycemia itself is a potent stimulus for islet cell hyperplasia (King & Chick 1976). In the transgenic mice, cellular proliferation, as measured by BrdU labeling, was significantly increased compared with wild-type mice. In both experimental models of type 2 diabetes and in diabetic subjects, maternal hyperglycemia, by providing an enhanced glucose load to the fetus, can result in islet hyperplasia (Suza et al. 1979). Since the transgenic mice are exposed to a hyperglycemic maternal environment in utero, a component of the increase in islet size and neonatal hyperinsulinemia may have been attributable to this phenomenon. The experiment where transgenic blastocysts were transferred to non-transgenic foster mothers was designed to address this point. Since pancreatic insulin content was similar in transgenic pups born to wild-type and transgenic foster mothers, the maternal uterine environment does not appear to be critical for the increase in islet number or pancreatic insulin content in transgenic mice.

Islet cell proliferation was increased in newborn transgenic mice. Although the blood glucose concentration in newborn transgenic mice was higher than in wild-type mice, this difference was not statistically significant. Since hyperglycemia is a potent stimulus for islet cell proliferation in early fetal and neonatal life, the increase in islet size and number observed in transgenic mice may be a result of the fetal hyperglycemia, as a consequence of the

Figure 3 Islet cell proliferation in wild-type and transgenic mice. Photomicrographs show representative results of BrdU labeling of islet cells from transgenic and wild-type 1-day-old mice. No staining was seen in newborn mice which had not been injected with BrdU. The histogram shows the percentage of labeled islet cells in each group of mice. The data represent the mean ± s.e.m. for four mice per group. Between 437 and 1760 cells were scored in each mouse. Magnification × 400.
neutralization of the hypoglycemic action of free IGF-I and II. Alternatively, overexpression of IGFBP-1 may actually enhance the mitogenic action of the IGFs on islets. While an inhibitory effect of IGFBP-1 on IGF-I action is most easily observed, IGFBP-1 can actually enhance IGF-I action under certain circumstances (Elgin et al. 1987), possibly due to facilitating transport of IGF-I to cell surface receptors. The phosphorylation status of IGFBP-1 has been shown to affect its affinity for IGF-I and to modulate its biological action (Jones et al. 1991). Indeed Hogg et al. (1993) have demonstrated in vitro synergism between IGF-I and IGFBP-1 in terms of fetal rat islet DNA synthesis using human IGFBP-1 from amniotic fluid. The phosphorylation status of the circulating IGFBP-1 in the serum or locally expressed IGFBP-1 in the islets of transgenic mice is not known. IGF-I is expressed in the pancreatic islets whereas the expression of IGF-II in islets is controversial. IGF-I immunoreactivity is localized to $\alpha$ cells whereas IGF-II immunoreactivity is localized to $\beta$ cells (Reinecke et al. 1993). Two points are of relevance to this spatial localization of the IGFs in the rodent pancreas. First, in the transgenic mice there was a relative increase in $\beta$ cells and a relative decrease in $\alpha$ cells. Secondly, the hyperglycemia, glucose intolerance and growth retardation (Rajkumar et al. 1995, 1996a) that develops in this transgenic model is only apparent after the first few weeks of life, at a time when expression of IGF-II and serum levels are declining. The significance of this temporal relationships remains to be determined. Consistent with previous reports in rodents, BrdU labeling was minimal after the neonatal period in both groups of mice. Islet cell replicative capacity is rapidly lost after the first few weeks of life, presumably due to depletion of the stem cell population (Swenne 1983). It is possible that this decline in islet proliferative capacity limits the ability of transgenic mice to compensate for the hyperglycemia that results from overexpression of IGFBP-1. Interestingly, apoptosis was also enhanced in islet cells from transgenic effects. This was observed in newborn mice where hyperglycemia was not evident. While hyperglycemia, per se does not appear to be the explanation for the enhanced apoptosis in the transgenic mice, the islets from transgenic mice were clearly under trophic stimulation as is evident by the higher serum insulin levels, increased cell proliferation and increased islet size and number. Increased apoptosis may be directly related to this excessive trophic stimulation or alternatively may be related to inhibition of IGF-I. IGF-I has been shown to inhibit apoptosis and to function as a survival factor for certain cell types in vitro (Cohick & Clemmons 1993). Further studies are required to determine whether this is the case for pancreatic islet cells. The data presented here indicate that there are changes in the islets of transgenic mice very early in development which partially compensate for the perturbation in glucose homeostasis that result from IGFBP-1 overexpression. In the neonatal period and presumably during fetal life, there is enhanced islet cell proliferation and increased apoptosis, although the overall result is larger

Figure 4 Apoptosis in islets from transgenic and wild-type mice. Photomicrographs show representative results for TdT labeled islet cells from wild-type and transgenic 1-day-old mice. Positive and negative controls as indicated in the Materials and Methods section are also shown. The histogram shows the percentage of apoptotic islet cells in each group of mice. The data represent the mean ± S.E.M. for three mice in each group. Approximately 800 islet cells from each group were scored. Magnification × 400.
and more numerous islets in transgenic mice. While these compensatory changes are sufficient to maintain normoglycemia in the early post-natal period there is a gradual decompensation, which is particularly marked at around the time of weaning and thereafter. Further studies utilizing this transgenic mouse model may provide some insight into the pancreatic dysfunction which occurs in type II diabetes.

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