Islets 3:5, 231-233; September/October 2011; ©2011 Landes Bioscience

Histopathology and ex vivo insulin secretion of pancreatic islets in gestational diabetes

A case report

Mariella Tancredi,[1] Lorella Marselli,[1] Cristina Lencioni,[1] Matilde Masini,[2] Marco Bugliani,[1] Mara Suleiman,[1] Pellegrino Masiello,[2] Ugo Boggi,[3] Franco Filipponi,[4] Francesco Dotta,[5] Piero Marchetti[1,6,†,*] and Graziano Di Cianni[1,†]

[1]Department of Endocrinology and Metabolism; [2]Department of Experimental Pathology; [3]Division of General Surgery and Transplantation in Uremia and Diabetes; [4]Liver Transplant Unit; [6]Unit of Endocrinology and Metabolism of Transplantation; University Hospital; Pisa, Italy; [5]Diabetes Unit; University of Siena; Siena, Italy

[†]These authors contributed equally to this work.

Key words: gestational diabetes, pancreatic islets, insulin secretion, apoptosis, regeneration

Gestational diabetes (GD) results from insufficient endogenous insulin supply. No information is available on features of islet cells in human GD. Herein, we describe several properties of islets from a woman with GD. Immunohistochemical stainings and EM analyses were performed on pancreatic samples. Islet isolation was achieved by enzymatic dissociation and density gradient centrifugation. Ex vivo insulin secretion was studied in response to fuel secretagogues. Control islets were obtained from matched non-pregnant, non-diabetic women. Total insulin positive area was lower in GD, mainly due to the presence of smaller islets. β-cell apoptosis and the presence of Ki67 positive islet cells were similar in GD and controls, whereas the amount of insulin positive cells in or close to the ducts was decreased in GD. Ex vivo insulin secretion did not differ between GD and non-pregnant, non-diabetic islets. These findings suggest that in this case of human GD there might mainly be a defect of β-cell amount, not due to increased apoptosis, but possibly to insufficient regeneration.

Introduction

Gestational diabetes (GD) is the type of diabetes with onset or first recognition during pregnancy.[1,2] Like all forms of hyperglycemia, it results from an endogenous insulin supply that is inadequate to meet insulin demands. This is well demonstrated in late pregnancy, when insulin requirements are uniformly high and differ only slightly between normal and GD women, whereas insulin secretion is significantly lower in GD.[3] The mechanisms regulating the adaptative changes of the β-cells during pregnancy are still unclear. In rodent models, it has been shown that β-cell hypertrophy and proliferation increase during pregnancy, return to pre-pregnancy levels shortly before parturition and decrease after delivery.[4,5] In addition, there is a marked increase of β-cell apoptosis post-partum, strongly contributing to the reduction of β-cell mass.[4,5] Very limited information is available in human beings. Autopsy studies have shown that during human pregnancy the amount of β-cells is significantly increased,[6,7] most likely due to enhanced neogenesis.[7] In this regard, it has to be kept in mind that, unlike in rodents,[8] no direct method is available in humans to assess regeneration phenomena. Since so far no information is available on β-cell features in human GD, herein we report the case of a woman with GD who died at the 27th week of gestation (wog). Pancreas histological analysis was performed, islets were isolated and ex vivo insulin secretion was evaluated.

Results

Figure 1 shows examples of the insulin immunohistochemistry results. The overall pancreatic section area that was analyzed was 187 ± 96 mm^2 (mean ± SD). Total insulin positive area per mm^2 was approximately 30% lower ($p < 0.01$) in the GD case (0.44 ± 0.10%, nine slides analyzed) than in NP-ND (0.69 ± 0.15%, 18 slides analyzed), mainly due to reduction of the area calculated for islets with a diameter ≥50 μm (representing more than 90% of the overall insulin area). However, average insulin positive area per islet was about 20% higher ($p < 0.01$) in GD (64.0 ± 12%, number of islets: 109) than in NP-ND (54 ± 13%, number of islets: 423), and the number of islets with a diameter ≥50 μm was similar in GD (0.90 per mm^2) and NP-ND (0.92 and 1.03 per mm^2). All this suggested differences in the average islet size. In fact, mean islet diameter was lower ($p < 0.01$) in GD (103 ± 31 μm) than in NP-ND (151 ± 71 μm). Glucagon positive area in the islets was similar in GD (29 ± 4%) and NP-ND (24 ± 6%).

*Correspondence to: Piero Marchetti; Email: piero.marchetti@med.unipi.it
Submitted: 10/28/10; Revised: 06/03/11; Accepted: 06/04/11
DOI: 10.4161/isl.3.5.15940

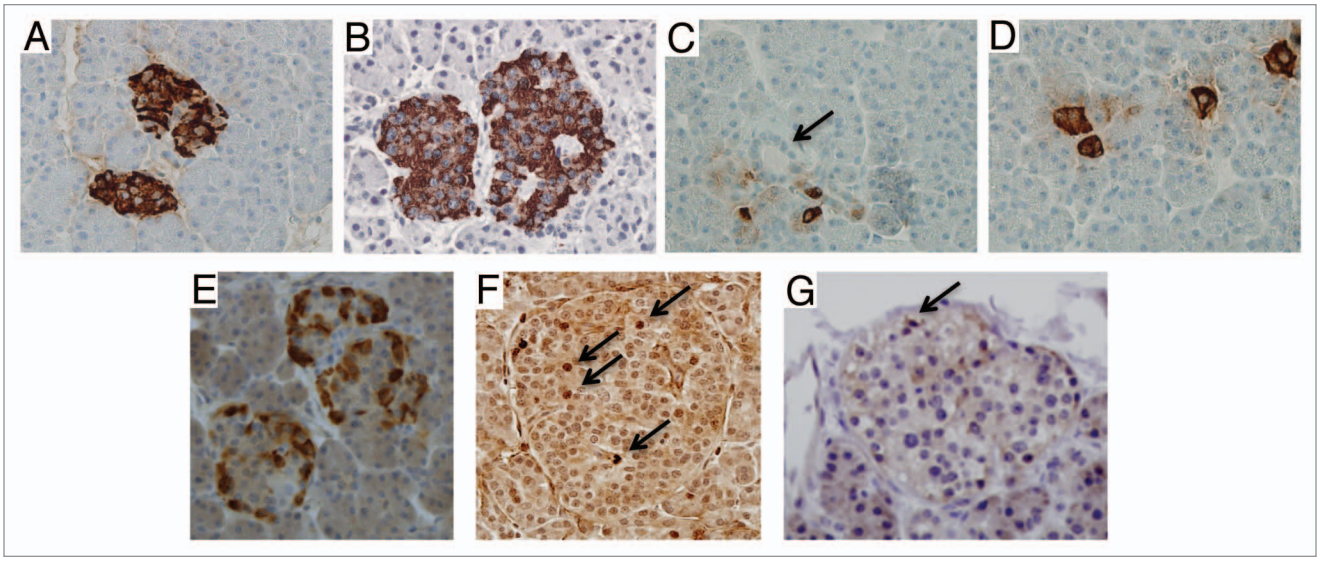

Figure 1. (A and B) show representative islets from the gestational diabetes (GD) case and control, respectively; as detailed in the text, islets in GD were smaller but with well maintained insulin positive areas. (C and D) show examples of insulin positive cells close to a duct (arrow) or scattered in the acinar tissue, respectively; as reported in the text, the former were less represented, and the latter more represented in the GD case. (E–G) show examples of glucagon, Ki67 (arrows) and activated caspase 3 (arrows) staining, respectively. Magnification x40.

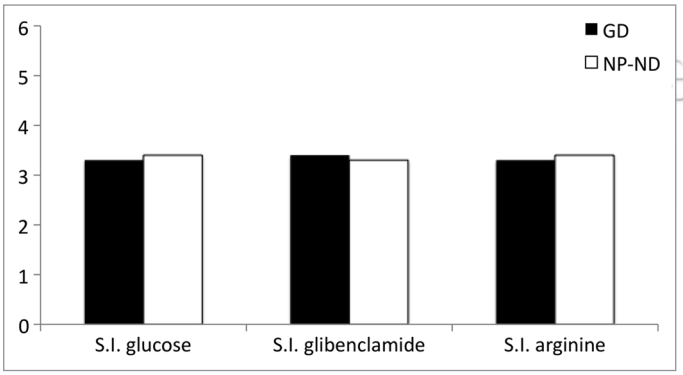

Figure 2. Stimulation index (S.I.) in response to glucose, glibenclamide and arginine of islets isolated from the gestational diabetes case (GD) and from non-pregnant, non-diabetic cases (NP-ND).

Apoptosis was evaluated by light microscopy by staining for cleaved caspase-3 (quantification was expressed as number of stained cells per islet) and by electron microscopy (quantification was expressed as percentage of apoptotic β-cells over the number of all the β-cells counted). Apoptosis was not increased in GD (cleaved caspase 3: 0.01 ± 0.00 positive cells per islet; EM: 1.0 ± 0.6%) compared to non-pregnant non-diabetic cases (cleaved caspase 3: 0.03 ± 0.00 positive cells per islet: EM: 1.3 ± 0.4%). Ki67 staining, used as replication marker, revealed 0.03 ± 0.00 and 0.04 ± 0.01 positive cells per islet in GD and NP-ND, respectively.

Ducts with insulin positive cells in the wall and/or at a distance within five nuclei from the wall were similarly represented in GD (34 out of 127, 27%) and NP-ND (104 out of 315, 33%). However, insulin positive cells in or near ducts was significantly ($p < 0.01$) lower in GD (3.6 ± 4.0) than in NP-ND (9.4 ± 13). Small insulin positive cell clusters (<10 cells) were more represented in GD (1.30 ± 0.5 per mm^2) than NP-ND (0.24 ± 0.05 per mm^2, $p < 0.01$).

Ex vivo insulin secretion (μU/islet/min) did not differ between the GD (three batches of 15 islets) and NP-ND (six batches of 15 islets) cases. It was respectively 0.03 ± 0.01 and 0.03 ± 0.01 at 3.3 mmol/l glucose; 0.099 ± 0.04 and 0.100 ± 0.05 at 16.7 mmol/l glucose; 0.102 ± 0.02 and 0.100 ± 0.04 in response to glibenclamide and 0.098 ± 0.02 and 0.103 ± 0.03 in response to arginine. The respective stimulation index values (stimulated insulin release over basal insulin release) were also similar (**Fig. 2**).

Subjects and Methods

RV was a 33 year old woman who died at the 27th wog from a cerebral hemorrhage due to rupture of a congenital aneurysm. GD was diagnosed based on glucose challenge at the 22nd wog. History was negative for the presence of diabetes in first degree relatives and for smoking. AntiGAD and antiIA2 autoantibodies were negative. Insulin therapy was initiated, with achievement and maintainance of good glycemic control (last evaluation: insulin dose 32 UI per day; fasting plasma glucose 104 mg/dl; HbA1c 6.1%). After death, the pancreas was processed with the approval of the local Ethics Committee. Samples for histology were taken at the level of the neck of the gland (the remaining tissue being processed for isolation of the islets) and processed for light and electron microscopy (EM) as previously described in references 9 and 10. Sections from three blocks for each pancreas were studied, using the following antibodies for immunohistochemistry: Guinea Pig anti-Insulin Concentrate (Invitrogen, Carlsbad, CA); Polyclonal Rabbit Anti-Human glucagon (Dako, Carpinteria, CA); Human/Mouse Cleaved Caspase-3 (Asp175) MAb (Clone 269518), Rabbit IgG (R&D Systems, Minneapolis, MN); Monoclonal Mouse Anti-Human Ki67 Antigen clone:Mib1

(Dako). Each section was 4 μm thick, and approximately 40 sections were cut for each block. Sections 10, 20 and 30 of each block were used for insulin staining, 12, 22 and 32 for glucagon, 14, 24 and 34 for Ki67, and 16, 26 and 36 for caspase-3. Upon electron microscopy, dead β-cells were identified on the basis of any of the following criteria: loss of plasma membrane integrity, fragmentation into discrete bodies, engulfment of cell corpse or its fragments by an adjacent cell.[11] The presence of marked chromatin condensation and/or blebs was considered to be a sign of apoptosis.[11] Islet isolation was performed by enzymatic digestion and density gradient purification and insulin secretion studies accomplished in response to glucose, glibenclamide and arginine during a 45 min incubation period, as previously reported in references 9 and 10. For insulin secretion studies, islets of similar size (diameter of around 150 μm) were used. Results were compared with those from islets of two non-pregnant, non-diabetic women (NP-ND) of comparable age (26 and 29 vs. 27 years) and body mass index (28.1 and 27.3 vs. 27.8 kg/m^2), who had died from trauma. Studying one case versus two cases does not allow statistical analysis. However, for the purposes of this report, data obtained from each slide of each block, and features of each single islet and insulin positive cell clusters were considered as separate observations.

Conclusions

In normal human pregnancy, the increased insulin demand due to reduced insulin sensitivity is mainly guaranteed by an increased β-cell amount[6,7] supposedly due to enhanced β-cell neogenesis from ducts and acinar tissue.[7] In our GD case, with death occurring at the 27th week of gestation, pancreatic insulin positive area was significantly decreased, compared to non-pregnant, non-diabetic subjects. This was not due to lower islet β-cell content, but rather to the presence of smaller islets, a feature observed in normal pregnancy as well.[7] Since the amount of β-cells is significantly increased in non-diabetic normal pregnant women,[6,7] it is reasonable to assume that, at least in the case described here, there was a major β-cell amount deficit. This is likely to be the major defect in this GD case, since ex vivo insulin secretion was similar in GD and NP-ND islets.

According to a previous report in reference 7, we did not find any difference as for apoptosis and replication (as estimated by Ki67 staining) between islet β-cells of GD and NP-ND cases. In addition, the use of electron microscopy allowed us to exclude the significant presence of other forms of cell death in GD islets, such as autophagic cell death.[11] However, apoptosis and regeneration phenomena are transient and rapid events, sometimes occurring as waves of death and proliferation. Therefore, we can not exclude that changes were not detected when studying a case at a single timepoint. In our GD case, the presence of insulin positive cells in and/or close to the duct walls was reduced, whereas the amount of insulin positive cells dispersed as small clusters in the acinar tissue was increased. Some authors consider the former as a surrogate marker of neogenesis from ducts and the latter from acinar tissue.[7,12] If so, in our gestational diabetes case there might have been a decreased neogenesis from ducts. However, at odds with rodents,[8,13] in humans no method is available to directly trace the origin of the β-cells.

Therefore, although limited to one only case, our findings suggest that, compared to normal pregnancy,[7] in gestational diabetes there might mainly be a defect of β-cell mass, not due to increase apoptosis, but to reduced β-cell regeneration. This scenario is partially different from that of type 2 diabetes, mainly characterized by increased β-cell death by apoptosis or other forms of programmed cell death and reduced β-cell function.[12,14] Specific hormonal factors in pregnancy could play a role in this regard.[15]

Acknowledgments

The study was performed within the framework of the EC funded project EURODIA (LSHM-CT-2006-518153).

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33:62-9.
2. Reece EA, Leguizamón G, Wiznitzer A. Gestational diabetes: the need for a common ground. Lancet 2009; 373:1789-97.
3. Buchanan TA, Xiang A, Kjos SL, Watanabe R. What is gestational diabetes? Diabetes Care 2007; 30:105-11.
4. Rieck S, Kaestner KH. Expansion of beta cell mass in response to pregnancy. Trends Endocrinol Metab 2010; 21:151-8.
5. Bernard-Kargar C, Ktorza A. Endocrine pancreas plasticity under physiological and pathological conditions. Diabetes 2001; 50:30-5.
6. Van Assche FA, Aerts L, De Prins F. A morphological study of the endocrine pancreas in human pregnancy. Br J Obstet Gynaecol 1978; 85:818-20.
7. Butler AE, Cao-Minh L, Galasso R, Rizza RA, Corradin A, Cobelli C, et al. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. Diabetologia 2010; 53:2167-76.
8. Bouwens L. Beta cell regeneration. Curr Diabetes Rev 2006; 2:3-9.
9. Masini M, Campani D, Boggi U, Menicagli M, Funel N, Pollera M, et al. Hepatitis C virus infection and human pancreatic beta-cell dysfunction. Diabetes Care 2005; 28:940-1.
10. Marchetti P, Bugliani M, Lupi R, Marselli L, Masini M, Boggi U, et al. The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. Diabetologia 2007; 50:2486-94.
11. Masini M, Bugliani M, Lupi R, del Guerra S, Boggi U, Filipponi F, et al. Autophagy in human type 2 diabetes pancreatic beta cells. Diabetologia 2009; 52:1083-6.
12. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52:102-10.
13. Bonner-Weir S, Inada A, Yatoh S, Li WC, Aye T, Toschi E, et al. Transdifferentiation of pancreatic ductal cells to endocrine beta-cells. Biochem Soc Trans 2008; 6:353-6.
14. Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M, et al. Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J Clin Endocrinol Metab 2004; 89:5535-41.
15. Devlieger R, Casteels K, Van Assche FA. Reduced adaptation of the pancreatic beta cells during pregnancy is the major causal factor for gestational diabetes: current knowledge and metabolic effects on the offspring. Acta Obstet Gynecol Scand 2008; 87:1266-70.