Pancreatic alpha-cells and insulin-deficient diabetes.

Short title: Glucagon and diabetes.

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Abbreviations. T1D: Type 1 diabetes; STZ: streptozotocin; FACS: fluorescence activated cell sorting.

Disclosure Summary: The authors have nothing to disclose.

Glucagon plays a major role in glucose homeostasis. This pancreatic hormone acts primarily on the liver activating gluconeogenesis and glycogenolysis, which promote hepatic glucose output, and increases plasma glucose levels (1, 2). While glucagon secretion by pancreatic alpha-cells is inhibited at high glucose concentrations, this process is augmented at low glucose levels (3). In this manner, glucagon release is one of the main lines of defense against hypoglycemia (4, 5). The complementary function of glucagon and insulin and their different regulation by nutrients and other control signals allow for the maintenance of plasma glucose levels within physiological ranges. Furthermore, glucagon stimulates hepatic fatty acid oxidation and ketogenesis, regulates food intake by central actions, increases adipose tissue thermogenesis and opposes to several insulin actions (2, 6).

In addition to its role in glucose homeostasis, a growing body of evidence indicates that glucagon is also involved in the pathophysiology of diabetes and some of its complications. Hyperglucagonemia, either absolute or relative to plasma insulin levels, has been related with increased hepatic glucose output in type 1 diabetes (T1D), which aggravates hyperglycemia. Despite the high plasma glucose levels present in diabetic individuals, glucagon secretion is not suppressed (4). In this regard, several therapeutic designs involve the decrease of glucagon secretion from pancreatic alpha-cells and/or the attenuation of glucagon actions on peripheral tissues (7). With the progression of T1D, the ability of pancreatic alpha-cells to respond to hypoglycemia becomes impaired, leading to defective counterregulation to falling plasma glucose levels (4, 5). This is a life-threatening situation, particularly in those diabetic patients subjected to insulin treatment (iatrogenic hypoglycemia) (8). All these functional defects in glucagon

secretion in T1D have been related with the lack of intraislet insulin signaling in pancreatic alpha-cells, intrinsic alpha-cell glucose-sensing defects and/or altered neural regulation of glucagon release (2, 8). However, the specific nature of these functional defects is still not well defined. The dynamics of the pancreatic alpha-cell mass also seems to play an important role to maintain absolute or relative hyperglucagonemia in diabetes. While T1D leads to specific immunological attack and destruction of pancreatic beta-cells, which results in decreased beta-cell mass, alpha-cell mass has been reported to be invariable or slightly increased in different models of autoimmune and insulin-deficient diabetes (9-11). This survival ability of pancreatic alpha-cells in a T1D environment may be probably related with their better autonomous immune responses (12) and survival gene networks (13) compared with beta-cells.

Despite the importance of pancreatic alpha-cells and glucagon secretion in glucose homeostasis and diabetes, the research about the physiology of this islet population has been neglected for a long time. In part, this minor attention has been due to the central role of pancreatic beta-cells in the pathogenesis of diabetes, which has been the focus of the majority of islet biology research. The study on alpha-cells was also hampered because of the lack of physiological identification patterns to recognize this islet cell type. Additionally, the number of pancreatic alpha-cells within the islet are scarce in rodents (15-20% of total cells), the main animal models used in diabetology (14). All these factors together with the difficulties to separate alpha from non-alpha-cells in enriched samples as well as limitations of conventional techniques have been a further restriction for a deeper exploration of this islet cell type. However, since the nineties, numerous technical advances have allowed to overcome these problems and to undertake

in depth studies on the regulation of glucagon secretion in health and diabetes at molecular and cellular levels. Among other valuable techniques, the employment of different transgenic mice with a florescent tag in glucagon-producing cells has been a major advantage for the identification, separation and characterization of living alpha-cells (15).

In this issue of *Endocrinology*, Dusaulcy et al. (16) used Glucagon-Venus transgenic mice, which express a fluorescent reporter in glucagon-containing cells (17). These animals received streptozotocin (STZ) to generate an insulin-deficient diabetic model. Part of them were also chronically treated with insulin using subcutaneous implants to analyze whether this hormone was able to correct the alterations. In this elegant study, the authors separated alpha-cells from non-alpha cells with high efficiency using fluorescence activated cell sorting (FACS). The use of purified samples of glucagon-containing cells was an important methodological issue, since it allowed the characterization of fundamental genes involved in alpha-cell biology. In agreement with previous studies (18), Dusaulcy et al. observed that STZ-treated mice developed hyperglycemia, hyperglucagonemia and increased pancreatic glucagon protein content, while no changes were observed in alpha-cell mass. Perifusion pancreas experiments showed the lack of suppression of glucagon secretion in response to high glucose concentrations, which is a feature frequently present in T1D (4). Importantly, experiments with FACS-sorted alpha-cells demonstrated that the majority of alterations in both plasma glucagon levels and hormone release from the pancreas were due to intrinsic changes in alpha-cell function: increased glucagon biosynthesis, hypersecretion and lack of glucose regulation. *In vitro* acute application of insulin was able to reduce

alpha-cell hypersecretion. While *in vivo* chronic treatment resulted in partial recovery of glucose-induced inhibition of glucagon secretion, this protocol did not reversed hyperglucagonemia. These results further indicate that impaired glucagon release in insulin-lacking diabetes is mainly due to effects of insulin deficiency and alterations in the intrinsic glucose-sensing capacity of the alpha-cell rather than alterations in neural regulation of pancreas function (1).

Dusaulcy et al. (16) also characterized in sorted alpha-cells the expression level of multiple genes relevant for alpha-cell biology. They showed that insulin-deficient diabetes leads to molecular alterations in several genes involved in glucose transport, glucagon synthesis, exocytosis, alpha-cell identity and insulin signaling. It was also shown that *in vivo* insulin treatment in STZ-treated mice could reverse some of the observed changes at the gene expression level. Interestingly, the glucose transporter Glut1 was one of the genes that was down-regulated in diabetic mice, and insulin treatment normalized its expression. Although this altered gene expression is likely to be involved with the defects found in glucagon secretion in the STZ-treated mice, it would have been interesting to analyze the state of other glucose sensitive proteins like AMPK and PASK, which have been identified as important regulators that link glucose metabolism to glucagon secretion in the alpha-cell (19, 20). These novel findings indicate that alpha-cell gene expression is really sensitive to the diabetic milieu, and it is very likely that these alterations affect numerous functions beyond glucagon secretion, like alpha-cell identity. Moreover, although insulin did not correct all the changes, these findings further confirm that insulin is a master regulator of the pancreatic alpha-cell in health and in diabetes.

In summary, the article of Dusaulcy et al. reveals important alterations in the pancreatic alpha-cell in a condition of insulin-deficient diabetes. These changes include glucose sensing, glucagon secretion and expression of numerous genes that are fundamental in the alpha-cell function. This study also opens new questions and research lines. For instance, it would be important to analyze whether the changes in alpha-cell gene expression are correlated with protein and function alterations. Also, a time course examination would be necessary to understand whether these gene expression changes are the cause or the consequence of the functional alterations in the alpha-cell, or they are an adaptive response to the stress environment generated by the STZ treatment. Finally, although the chemical beta-cell ablator STZ is often used as a T1D model, it would be interesting to explore similar parameters as those shown by Dusauley et al. in other models that better recapitulate the steps and characteristics of human autoimmune T1D, like NOD (non-obese diabetic) mice and bio-breeding diabetic-prone rats (21, 22).

Aknowledgements. This work was supported by grants from the Ministerio de Ciencia e Innovación (BFU2013-42789-P) and Generalitat Valenciana (PROMETEOII/2015/016). CIBERDEM is an initiative of the Instituto de Salud Carlos III.

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