Chapter 1
Microscopic Anatomy of the Human Islet of Langerhans

Peter In't Veld and Miriam Marichal

Abstract Human islets of Langerhans are complex micro-organs responsible for maintaining glucose homeostasis. Islets contain five different endocrine cell types, which react to changes in plasma nutrient levels with the release of a carefully balanced mixture of islet hormones into the portal vein. Each endocrine cell type is characterized by its own typical secretory granule morphology, different peptide hormone content, and specific endocrine, paracrine, and neuronal interactions. During development, a cascade of transcription factors determines the formation of the endocrine pancreas and its constituting islet cell types. Differences in ontogeny between the ventrally derived head section and the dorsally derived head, body, and tail section are responsible for differences in innervation, blood supply, and endocrine composition. Islet cells show a close topographical relationship to the islet vasculature, and are supplied with a five to tenfold higher blood flow than the exocrine compartment. Islet microanatomy is disturbed in patients with type 1 diabetes, with a marked reduction in β-cell content and the presence of inflammatory infiltrates. Histopathological lesions in type 2 diabetes are less pathognomonic with a more limited reduction in β-cell content and occasional deposition of amyloid in the islet interstitial space.

Keywords Pathology · Type 1 diabetes · Type 2 diabetes · Morphology · Anatomy · Insulitis · Amyloid · β-cell · α-cell · δ-cell · PP cell · Autoimmunity · Innervation · Vasculature · Non-endocrine cells

1.1 Introduction

The human pancreas is an unpaired gland of the alimentary tract with mixed exocrine–endocrine function. It is composed of four functionally different, but interrelated components: the exocrine tissue, the ducts, the endocrine cells, and

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the connective tissue. These elements are intimately related through ontogeny, anatomy, histology, and function. Because the scope of this chapter is the microscopic anatomy of the islet of Langerhans, the other components will only briefly be mentioned.

1.2 The Islets of Langerhans

The pancreas has an elongated shape, and somewhat resembles a 17th century pistol with a curved handle and thick barrel. The handle is formed by the head of the gland, which is closely attached to the distal two-thirds of the duodenum, the barrel is formed by the body region, which is overlaid by the posterior wall of the stomach, and by the tapering tail region that ends near the splenic hilus. Macroscopically, the pancreas has a yellowish-pink aspect and a soft to firm consistency depending on the level of fibrosis and fat accumulation in the organ. It has an average weight of 68 g (range 45–120 g) [1] and is composed of small lobules measuring 1–10 mm in diameter. Microscopically, the lobules are formed by a mixture of ductules and well-vascularized epithelial cell clusters that reflect the two main functions of the pancreas: digestion and glucose homeostasis. Exocrine cells (98% of the parenchyma) release a mixture of digestive enzymes and bicarbonate into the duodenum. They are organized into acini that open into intercalated ducts, to which they are connected via centro-acinar cells. The intercalated ducts fuse into intralobular ducts, interlobular ducts, and finally into the main pancreatic ductus of Wirsung, which together with the common bile duct, opens into the duodenum at the papilla of Vater (papilla major). The secondary ductus of Santorini ends in the papilla minor, a few centimeters above the papilla major. Endocrine cells (1–2% of the parenchyma) release nutrient-generated hormones into the portal vein. Clusters of endocrine cells form islets of Langerhans, micro-organs that lie scattered throughout the exocrine parenchyma in between the acini and ductal structures. The islets of Langerhans are of vital importance to the body as they produce insulin, a prime regulator of glucose homeostasis. The name 'islets of Langerhans' was coined by Edouard Laguesse (1861–1927), a histologist working at the University of Lille, who, in a seminal paper in 1893, correctly deduced that they are involved in endocrine secretion. He named them after Paul Langerhans (1849–1888), who was the first to describe these cell clusters in his doctoral thesis in 1869 but who was unable to attribute them with a specific function [2]. The adult human islet of Langerhans has a mean diameter of 140 μm [3]. It is pervaded by a dense network of capillaries [4] and is (partly) surrounded by a thin collagen capsule [5] and glial sheet [6] that separates the endocrine cells from the exocrine component. Islets vary in size and range from small clusters of only a few cells to large aggregates of many thousands of cells. Depending on the exact manner in which an 'islet' is defined, the estimate of islet number in the adult human pancreas varies from several hundred thousand to several million. Total beta mass appears to be highly variable between subjects, ranging from 500 to 1500 mg [7], corresponding to an estimated 10^9 β-cells and 1–2% of

mean pancreatic weight. Adult islets contain four major endocrine cell types: α-cells (also referred to as A-cells), β-cells (also referred to as B-cells), δ-cells (D, formerly also called A1), and PP cells (pancreatic polypeptide cells, formerly also called F or D1 cells). A fifth cell type, the Epsilon or Ghrelin cell has recently been described.

1.3 Embryology and Fetal Development

The pancreas is derived from two primordia in the distal embryonic foregut [8, 9]. At 3–4 weeks of gestation, a dorsal primordium is formed opposite the hepatic diverticulum and a ventral primordium (sometimes bi-lobed) in close apposition to the diverticulum. At 6 weeks of gestation the ventral pancreas rotates, and fuses with the dorsal pancreas around week 7. The ventral primordium gives rise to part of the head region of the gland ('ventral head'), while the dorsal primordium gives rise to the dorsal head, the body, and the tail. This difference in ontogeny is reflected in significant differences in endocrine cell composition, vascularization, and innervation between the ventral and dorsal pancreas. The ventral head is drained of exocrine secretion by the ductus of Santorini and is supplied with blood via the mesenteric artery. The dorsally derived head, body, and tail are drained by the ductus of Wirsung and irrigated by the coeliac artery. The differences in ontogeny are mirrored by differences in islet composition [10, 11].

Pancreas development is controlled by a complex cascade of transcription factors [12]. Pancreatic and duodenal homeobox 1 (Pdx1) induces early (primary) progenitor cells to expand and form duct-like outgrowths into the surrounding mesenchyme. In a second wave of differentiation (secondary transition), cells at the duct tips differentiate into acini, and cells in the duct walls give rise to endocrine cells, a process driven by another key transcription factor Neurogenin3 (Ngn3). Endocrine cells are first detected at 8–9 weeks at the basal side of the ductal epithelium where they grow out to primitive islets. Exocrine acini are observed from 10 to 12 weeks. Growth of the endocrine mass during fetal life follows that of the total gland, with endocrine tissue forming 2–5% of the parenchyma [13]. Growth of β-cell mass in fetal and adult life appears to be partly by neogenesis from endogeneous Ngn3+ progenitor cells [14] and partly by replication of existing β-cells. β-cell replication peaks around 20 weeks of gestation after which replication levels decrease exponentially reaching near zero values a few years after birth [15–17].

During early development the percentage of the various endocrine cell types changes: at 8 weeks approximately 50% of endocrine cells express glucagon, decreasing to 15–20% in the adult. Similarly, the percentage of D-cells decreases from 20 to 25% in neonates to approx 5% in adults [18–21].

1.4 Endocrine Cell Types

Adult human islets contain at least five different endocrine cell types. α and β-cells were both first described in 1907 by Lane [22] on the basis of their histochemical

staining characteristics, while D-cells were first recognized by Bloom in 1931 [23]. Both PP cells [24] and Ghrelin cells [25] were discovered with the aid of immunocytochemistry.

1.4.1 α-Cells

α-cells secrete glucagon, a 29-aminoacid peptide with hyperglycemic action [26]. The peptide is derived from proglucagon (180-aminoacids) through proteolytic cleavage. Other cleavage products that can be derived from the precursor are GLP-1, GLP-2, and glicentin [27, 28]. Glucagon is stored in secretory granules that have a typical morphology with an electrondense core and a grayish peripheral mantle [29]. Glucagon was immunohistochemically localized to the α-cells by Baum et al. [30]. The number of α-cells is estimated at 15–20% [31, 32], although the relative volume taken up by α-cells can vary significantly between islets with some islets containing up to 65% of α-cells [33]. α-cells are most prominent in the dorsally derived part of the pancreas and virtually absent in the ventrally derived part (Table 1.1).

1.4.2 β-Cells

β-cells form the bulk of the pancreatic endocrine cell mass. Depending on the morphometric techniques that were used, the type of samples analyzed, and the extent of the analysis, a relative islet β-cell mass was found between 50 and 80% [31–34]. β-cells secrete insulin, a 51-aminoacid peptide with strong hypoglycemic action. Insulin is essential for cellular nutrient uptake and thus for the survival of the organism. Its isolation and immediate successful clinical application in 1923 by Banting, Best, and Collip was one of the major medical breakthroughs of the 20th century [35, 36]. Like virtually all peptide hormones, insulin is proteolytically derived from a precursor molecule, proinsulin. This biologically inactive precursor is split into

Table 1.1 Cell types in the adult human endocrine pancreas

	Cell type				
	A	B	D	PP	Epsilon
Peptide hormone	Glucagon	Insulin	Somatostatin	Pancreatic polypeptide	Ghrelin
Molecular weight	3500	5800	1500	4200	3400
Number of amino acids	29	51	14	36	28
Volume % (adult)					
Dorsal	15–20	70–80	5–10	<1	1
Ventral	<1	10–20	2	80	1
Total	15–20	70–80	5–10	15–25	1

three parts, an A and a B chain, which remain connected by two sulfur bridges, thus forming the biologically active insulin molecule, and a C chain (Connecting peptide), which is released together with insulin in a 1:1 molar ratio [37]. The β-cell also co-secretes Islet Associated Polypeptide (IAPP, also called amylin), a 37-aminoacid peptide related to calcitonin gene related peptide (CGRP) [38]. Under pathological conditions IAPP molecules may polymerize and form large intraislet amyloid deposits that are characteristic for type 2 diabetes and for insulinoma.

Insulin was first immunohistochemically localized to the β-cell by Lacy [39]. It is stored in cytoplasmic secretory vesicles that have a characteristic morphology with an electrondense core and a clear peripheral mantle (Fig. 1.1). Within the 350 nm granule, insulin (but not proinsulin) is complexed to zinc, forming insulin–zinc hexamers and crystalline granule cores. Depending on the maturation stage of the granule, the mantle may contain unprocessed proinsulin; when the proteolytic enzymes (prohormone convertases PC1-2, carboxypeptidase-H) present in the newly formed secretory granule have not yet resulted in sufficient cleavage of the precursor molecules, the granule core may be absent and typical immature 'gray' granules are found [39]. The biological reason for Zn complexation is not well understood, but its presence is of practical benefit in islet isolation procedures, where zinc-chelating dyes like dithizone [40] are helpful in determining islet yield and purity.

A β-cell is estimated to contain 9–13.000 secretory granules [41, 42]. With an average daily insulin requirement of 40 IU and an average insulin content per granule of 8 fg, it can be estimated that approx 10^{12} secretory granules are released from β-cells each day. Release may occur via a nutrient-regulated pathway or via a constitutive pathway. Nutrient-induced release is initiated via closure of ATP-dependent

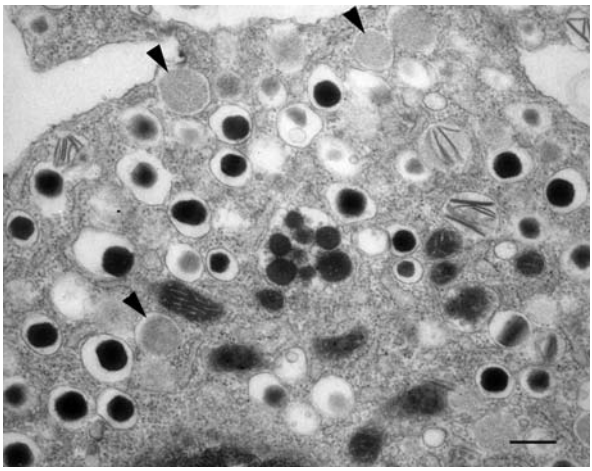

Fig. 1.1 Electron-microscopic image of an islet β-cell with mature dense-cored secretory granules and immature gray granules (*arrowheads*) (bar 300 nm)

potassium-channels, membrane depolization, opening of voltage-dependent calcium channels, and calcium-induced fusion of the secretory granules with the plasma membrane. The process of insulin release is complex and may partly consist of granule fusion with the plasma membrane and partly of temporary opening of small pores between the granule lumen and the extracellular milieu [43].

In addition to (pro)insulin, C-peptide, IAPP, zinc, and proteolytic enzymes, the secretory granule contains calcium, adenine nucleotides, biogenic amines, and a series of additional peptide (pro)hormones including chromogranin A and beta-granin [44, 45]. Several granule (membrane) proteins have been implicated in humoral autoimmunity in type 1 diabetes, like the zinc transporter ZnT8 [46], insulinoma-associated protein 2 (IA-2; ICA-512) [47], and glutamic acid decarboxylase (GAD65) [48].

β-cells in the human pancreas may show marked variation in granulation, cell size, and size of the nuclei (Fig. 1.2). Differences in granulation and cell size may reflect a heterogeneity in glucose responsiveness and biosynthetic activity [49], while differences in nuclear size may reflect polyploidy with nuclear DNA content of up to 8n being relatively common [50]. β-cells in the aging human pancreas display multiple prominent lysosomes with lipid-like content (Fig. 1.3). These strongly autofluorescent organelles resemble the lipofuscin inclusions in aging neurons and linearly increase with age [51].

1.4.3 D-Cells

The D (or δ) cells release somatostatin (formerly called somatotropin release inhibiting factor), first isolated from in the hypothalamus [52]. This peptide hormone is a

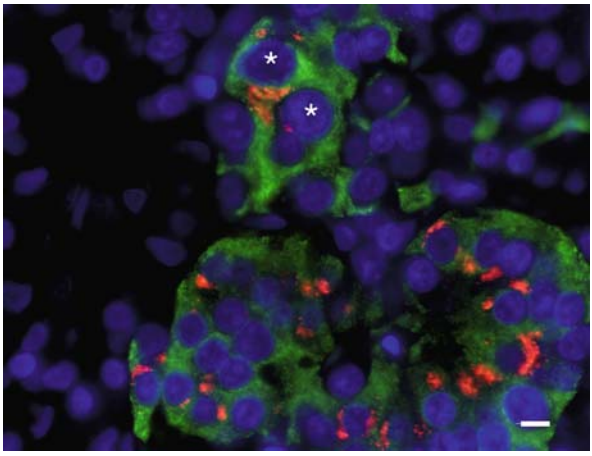

Fig. 1.2 Two-color fluorescent imaging for insulin (*green*) and proinsulin (*red*) of a human islet of Langerhans. Proinsulin has a predominantly perinuclear localization. Note the significant differences in nuclear size between islet β-cells (*asterix*) (Bar 10 μm)

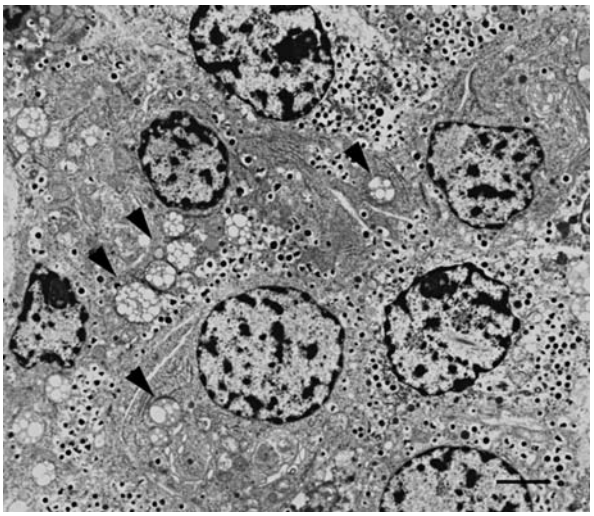

Fig. 1.3 Electron-microscopic image of aging human β-cells with multiple cytoplasmic inclusions (bar 5 μm)

potent inhibitor of glucagon and insulin release and was first immunohistochemically located to the D-cell by Luft et al. [53]. The hormone exists in a 14-aminoacid form and in a 28-aminoacid form [54]. Although all islet cells have neuron-like characteristics, the D-cells resemble small neurons most, as they often form long slender processes with a secretory-granule rich knob-like ending near a capillary suggesting focal and possibly paracrine secretion [55]. D-cells form 5–10% of islet volume (Table 1.1).

1.4.4 PP Cells

The least well studied of the islet hormones is PP, secreted by the PP cell. The peptide has been found immunocytochemically in two morphologically distinct cell types: PP immunoreactive cells (formerly designated as F-cells), characterized by round to angular secretory granules, were found in the ventrally derived head of the pancreas, while cells with small granules, formerly called D_1 cells, were found in the dorsally derived part [56]. In the human pancreas the relative PP cell mass in the ventral pancreas is considerable, constituting up to 80% of the cells (Table 1.1).

1.4.5 Epsilon Cells

The latest cell type that was added is the Epsilon or Ghrelin cell. The hormone ghrelin was first isolated from rat stomach and later localized to a specific cell type in the

adult human islet [25]. Adult islets contain less than 1% epsilon cells. The hormone is thought to be of importance in growth hormone release, metabolic regulation, and energy balance, but its exact role in islet cells has yet to be established.

1.5 Islet Anatomy

Endocrine cells in the pancreas form aggregates of various sizes and microscopic aspect. Larger aggregates, the islets of Langerhans, form small, ellipsoid or spherical structures dispersed throughout the exocrine part. The islet size and number of β-cells increases from birth to adulthood [16]. In fetuses, islets are in close contact with ducts, but they become more separated from the ducts in neonates and adults. In adults, 50% of the islets remain close to the ducts [57]. Size and distribution of islets vary widely from individual to individual, but without recognizable pattern, except that their number seems to increase towards the tail of the pancreas [58, 59]. On light microscopy, the epithelial cells of the islets of Langerhans form trabecular structures, separated by a dense network of anastomosing capillaries [4]. Two architecturally different types of islets are recognized: the diffuse and the compact islet. In the postero-inferior (ventral) head of the pancreas, the islets are of the 'diffuse' type, because the trabeculae seem more loosely arranged than in the islets occurring in the rest of the pancreas and which are known as 'compact islets'. The diffuse islets are very rich in PP cells and are larger than the compact islets. They also contain substantially less A, B, and D cells than the compact islets [60], which are primarily found in the body and tail and have sizes ranging from 50 to 280 μm. Compact islets are well circumscribed and separated by a thin layer of collagen from the surrounding acini. This is less the case in the diffuse islets, which are often irregular. Though occasional islets can measure 1–2 mm in diameter, compact islets larger than 250 μm are generally considered hyperplastic [61].

In humans, the endocrine cells are distributed throughout the islets without apparent organization; this contrasts with murine islets, which show a clear topographical separation of β and α-cell mass. It cannot be excluded that such topographical differences between human and rodent islets are paralleled by differences in endocrine and paracrine islet cell interactions. The cytoarchitecture of the human islet, with its random islet cell distribution, does not support functional islet domains in which the direction of blood flow determines intraislet endocrine signaling [34]. The relative proportion of the various endocrine cell types in the human islets can vary considerably; in one study [33] the percentage of β-cells ranged from 28 to 75%, that of α-cells from 10 to 65% and that of somatostatin cells from 1.2 to 22%. Not all endocrine cells in the pancreas occur in classical islet structures: 15% of all β-cells are found in units with a diameter of <20 μm (1–3 cells) and without associated glucagon, somatostatin, or PP cells [62]. These units, referred to as 'single β-cells' are equally distributed throughout the whole gland and in close association with acini and ductules; they are significantly smaller than β-cells located in larger islets. It has been speculated that these cells are an early stage in the formation of new

islets, although recent studies in rodents using β-cell lineage tracing were unable to confirm this [63].

The different islet cell types can be distinguished with special stains. Nowadays immunohistochemistry is used almost exclusively, but several cell-type-specific histochemical stains are available as well. The best known are Gomori's aldehyde fuchsin for β-cells [64, 65] and Hellman–Hellerström for δ-cells [66]. The Mallory-Azan stain distinguishes between the three major cell types.

1.6 Non-endocrine Islet Cells

Between the islet cell trabeculae, small amounts of connective tissue are present, with blood vessels being most prominent. Other non-epithelial elements present in the islet are nerve fibers, pericytes, macrophages [67], and dendritic cells; the latter express major histocompatibility complex (MHC) class II molecules on their cell surfaces, which may play a role in graft rejection and the initiation of type 1 diabetes.

Pancreatic lymphatics are found in the interlobular septa of the exocrine portion, but are seldom in contact with the islets [68].

1.7 Islet Vasculature

The islet vasculature is critical for adequate glucose homeostasis, not only because of the high oxygen consumption of pancreatic β-cells, but also because of timely responses to changes in plasma glucose concentration and the release of islet hormones into the circulation. Islet perfusion is mediated by neural, hormonal and circulatory signals [69]. The islet capillary network has a density five times higher than the exocrine capillary network [70, 71] and its vasculature is akin to the glomerular system of the kidney: 1 to 3 afferent arterioles provide the islet with oxygenated blood, which leaves through efferent venules; these empty into exocrine capillary networks or collecting venules that in turn empty directly into larger veins. Another similarity to glomeruli is that a variant of nephrin (a podocyte marker) has recently been shown to mark the islet vasculature [72]. The islet endothelium contains 95 nm fenestrations closed by a diaphragm and arranged into sieve plates (Fig. 1.4). Islet capillaries display up to tenfold more fenestrations than exocrine capillaries [73], further illustrating the close interaction between islet cells and the circulation. VEGF-A released from pancreatic β-cells was shown to be a determining factor in inducing islet capillaries and their fenestrated endothelial cells [74]. Islet β-cells are usually bordered by at least one capillary and show polarity in their cytoplasm with the secretory granules at the apical pole towards the blood vessel [75]. Islet capillaries are surrounded by a double basement membrane, each characterized by its own laminin subtypes. One basement membrane is derived from a peri-islet membrane that accompanies the capillary along its winding path throughout the islet; the

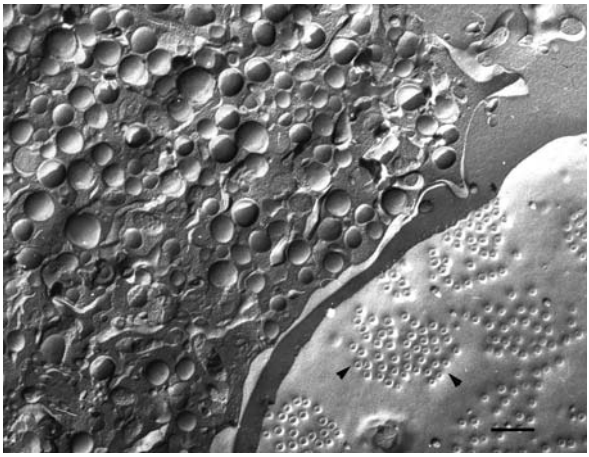

Fig. 1.4 Freeze fracture replica of a rat islet showing a fenestrated capillary with fenestrations arranged into sieve plates (*arrowheads*). Adjacent to the capillary is an endocrine cell with multiple secretory granules in the cytoplasm (bar 300 nm)

endothelial basement membrane constitutes the other. This situation differs from that in rodents where only a single basement membrane was found [76].

1.8 Innervation

Islets have sympathetic, parasympathetic, and sensory innervation; the nerve fibers contain acetylcholine, noradrenaline, and several neuropeptides. The fibers accompany the vasculature and are embedded in non-myelinating Schwann cells. They end blindly in the pericapillary space in close proximity to the islet cells; true synaptic contacts on islet cells have not been described but close nerve–islet cell interactions appear to be mediated by CADM1 (cell adhesion molecule 1) [77]. The ventral and dorsal parts of the pancreas have different innervation, with the dorsal pancreas receiving its sympathetic innervation from the celiac ganglion and the ventral pancreas from the superior mesenteric ganglion. Insulin secretion is stimulated by the parasympathetic system and inhibited by the sympathetic system [78]. It has been postulated that thin peri-islet Schwann cell sheets and sensory afferent neurons may play a role in the initiation of type 1 diabetes [79].

1.9 Islet in Type 1 Diabetes

Patients with recent onset type 1 diabetes (DM1) usually present with a pancreas that is macroscopically normal in appearance and weight. This contrasts with findings in patients with chronic disease in whom the lack of endogenously released insulin

leads to the atrophy of the acinar cells and a decrease in overall pancreatic weight [80, 81].

The characteristic lesion in recent onset DM1 is formed by the presence of inflammatory infiltrates in the islets of Langerhans. In a seminal study in 1965 [80], Willy Gepts described the presence of insulitis in 15/22 young patients with a duration of the disease of <6 months. He observed that the inflammatory lesions were limited to islets in which β-cells were still present and that most remaining islets were pseudoatrophic and contained only non-β-cells (Fig. 1.5), resulting in an overall decrease in β-cell mass to 10% of normal values. He concluded that DM1 was probably the result of a protracted inflammatory disease of autoimmune or viral etiology. Subsequent studies using immunohistochemical staining and precise morphometric methods have confirmed these initial histopathological findings [82], but the use of more sensitive techniques also indicated that residual β-cells are still present many years after clinical onset, especially in older individuals. Our knowledge of the disease processes leading to overt diabetes is still fragmentary due to the fact that only a few dozen cases of very recent onset diabetes could

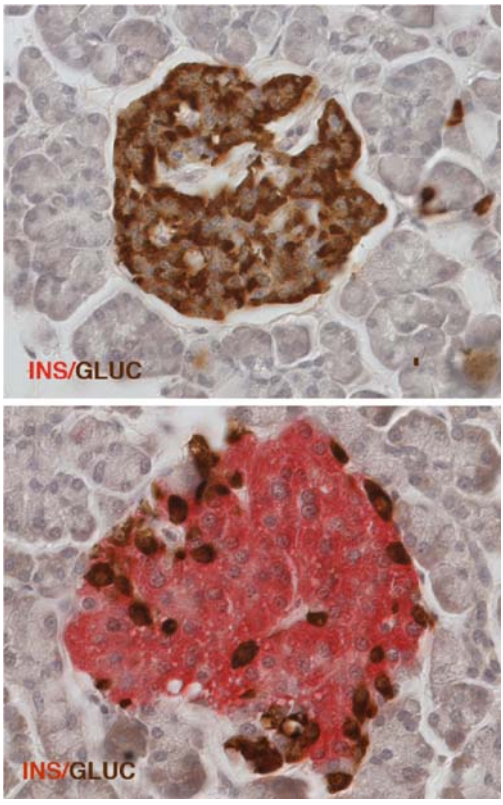

Fig. 1.5 Islets stained for insulin (*red*) and glucagon (*brown*). Islets from chronic type 1 diabetics are pseudoatrophic and consist primarily of α-cells (*top panel*), in contrast to islets from a normal control with both α and β-cells

be studied by autopsy and this often under conditions that precluded extensive molecular and immunological studies [83]. Our current understanding of the disease process indicates that a T-cell-mediated autoimmune reaction against islet β-cells occurs in genetically susceptible individuals and that this process appears to be initiated by environmental triggers [84]. The intensity of the disease process appears to vary between patients and is often more severe in children. At clinical onset, most patients still retain a significant β-cells mass (averaging 10–30% of normal values), but most islets have lost their β-cell component and only contain α, D-, and PP cells; these islets are usually referred to as pseudoatrophic [80, 83]. A small fraction of islets still contain both β-cells and non-β-cells in normal proportions. Such β-cell containing islets may contain an inflammatory infiltrate that predominantly consists of CD8-positive T-cells and macrophages [85, 86]. Neither the mechanism leading to the leucocytic infiltration is known, nor has the antigen toward which the immune response is directed been identified.

Studies of the early phases leading to overt diabetes have indicated that positivity for autoantibodies directed against islet cell antigens often predate the disease by many years. The presence of multiple autoantibodies in combination with a susceptible HLA-DQ genotype was shown to have a predictive value of >70% in relatives of DM1 patients [87]. As the effector phase of the disease appears to be cell mediated, the presence of autoantibodies may function as surrogate markers for islet cell destruction. Histopathological studies in non-diabetic adult organ donors with positivity for multiple autoantibodies and a susceptible HLA-DQ genotype showed that only a minor part (<10%) of the islets presented with insulitis or other histopathological lesions (Fig. 1.6). As such islets also showed high levels of β-cell replication, it cannot be excluded that the clinical outcome of autoimmune attack depends on

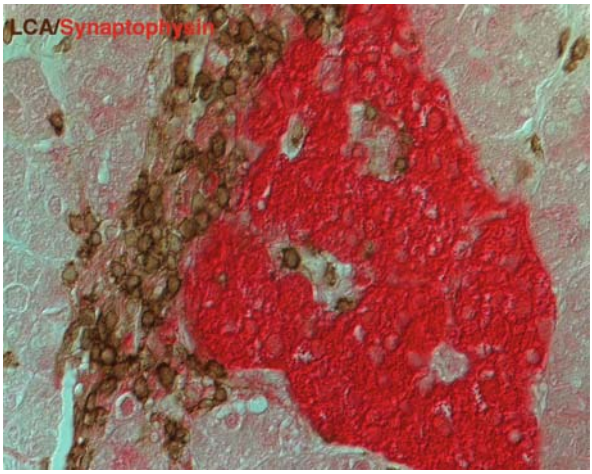

Fig. 1.6 Insulitis in an islet of Langerhans from a non-diabetic autoantibody-positive organ donor. Infiltrating leucocytes are stained with leucocyte common antigen (*brown*) and the islet cells are stained with the pan-endocrine marker synaptophysin (*red*)

the balance between β-cell replication and autoimmune β-cell destruction [88]. Evidence that such regenerative processes may also occur in young patients with recent onset of the disease is found in the early cases described by Gepts, where islet hyperplasia was observed in a 2-year-old child that died 60 days after diagnosis in ketoacidosis. In this patient a single lobe of the gland showed marked hyperplasia of insulin-containing islets in a pancreas that was devoid of β-cells in the remaining part [80]. Additional evidence that β-cell regeneration may play a role in disease progression comes from studies where β-cell apoptosis was found in patients with long-standing DM1 [89], indirectly suggesting that β-cells are still being replenished many years after the onset of the disease. The mechanism underlying β-cell regeneration in the diabetic pancreas is unknown and may either involve neogenesis or replication, although no evidence of β-cell replication was found in recent onset patients who died in ketoacidosis [90].

Although the bulk of the evidence favors an autoimmune etiology of the disease, it is likely that at least some cases of DM1 have a viral origin as the Coxsackie B4 enterovirus could be isolated from a small series of recent-onset DM1 patients characterized by a non-destructive islet inflammation consisting of natural killer cells [91].

1.10 Islets in Type 2 Diabetes

β-cells can adapt to a large number of physiologic stimuli: athletes secrete 2–3 times less insulin than normal individuals in order to reach normoglycemia [92]. Compared to lean non-diabetics, obese subjects can secrete 2–5 times more insulin in response to a glycemic challenge [93]. Pregnancy is another example in which insulin secretion rises drastically in response to physiologic demand [94, 95]. Type 2 DM occurs in predisposed individuals when the adaptive capacity of the endocrine pancreas fails. Several factors can contribute to this failure. DM2 is considered a disease of insulin resistance and insulin deficit, loss of β-cell mass, increased apoptosis, and amyloid deposition. Genetic and environmental factors also play an important role. There is no real histological ‘hallmark’ for type 2 diabetes in the human pancreas. Amyloid deposition comes closest to being such a ‘hallmark’, because the majority of type 2 diabetic subjects show deposition of non-AA amyloid in at least some of their islets. However, not all DM2 subjects show amyloid deposition and islet amyloid can be found in islets of non-diabetics [96–99]. The precursor of amyloid in DM2 is Islet Amyloid Polypeptide (IAPP) or amylin, a 37-amino acid peptide which is present in β-cell secretory granules, and is co-secreted with insulin [100, 101]. Its function is not known. The number of amyloid affected islets is not clearly related to the duration of diabetes in man [98, 102, 103], but may be related to the degree of insulin resistance and islet failure [104]. Affected islets are mostly found in the dorsal head, body, and tail and are rare in the ventral head [105, 106]. Islets located at the periphery of the pancreas exhibit a higher percentage of amyloid deposition than islets in the central regions [105]. The histochemical

staining properties of islet amyloid are the same as for the other forms of amyloid (Congo Red being the most specific stain). Immunohistochemistry for IAPP is another method to demonstrate islet amyloid. It is obvious from a morphologist's point of view that once islets are almost completely invaded by amyloid they can hardly function correctly and this can result in failure to secrete hormones into the blood stream and failure to get sufficient nutrients to the islet cells. However, the number of islets affected in this way is minimal in most diabetics and therefore this does not seem to play a major role in the pathogenesis of DM2 [107]. Most authors do agree that in DM2 the β-cell mass is reduced, but the reduction in β-cell mass early in the disease seems insufficient to cause diabetes in the absence of β-cell dysfunction [108, 109]. When amyloid causes β-cell loss in DM2, this is probably through membrane disruption caused by amyloid fibers. This hypothesis, known as the 'toxic oligomer hypothesis,' is based on findings in neurodegenerative diseases [110]. Since it has been shown that patients with Alzheimer disease are more prone to DM2 than non-Alzheimer patients [111], a link between both diseases is possible.

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