Unique arrangement of alpha- and beta-cells in human islets of Langerhans

Short running title: cell arrangement in human islets of Langerhans

Domenico Bosco, Mathieu Armanet, Philippe Morel, Nadja Niclauss, Antonino Sgroi, Yannick D. Muller, Laurianne Giovannoni, Géraldine Parnaud and Thierry Berney

Cell Isolation and Transplantation Center, Department of Surgery, Geneva University Hospitals and University of Geneva Rue Michel-Servet 1, 1211 Geneva 4, Switzerland

Corresponding author:
Domenico Bosco
E-mail: domenico.bosco@unige.ch

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org

Submitted 7 August 2009 and accepted 6 February 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
Objective: It is generally admitted that the endocrine cell organisation in human islets is different to that of rodent islets. However, a clear description of human islet architecture has not yet been reported. The aim of this work is to describe our observations on the arrangement of human islet cells.

Research Design and Methods: Human pancreas specimens and isolated islets were processed for histology. Sections were analysed by fluorescence microscopy after immunostaining for islet hormones and endothelial cells.

Results: In small human islets (40-60 μm-diameter), beta-cells had a core position, alpha-cells a mantle position and vessels laid at their periphery. In bigger islets, alpha-cells had a similar mantle position but were found also along vessels that penetrate and branch inside the islets. As a consequence of this organisation, the ratio of beta-cells to alpha-cells was constantly higher in the core than in the mantle part of the islets, and decreased with increasing islet diameter. This core-mantle segregation of islet cells was also observed in type 2 diabetic donors but not in cultured isolated islets. Three-dimensional analysis revealed that islet cells were in fact organized into trilaminar epithelial plates, folded with different degrees of complexity and bordered by vessels on both sides. In epithelial plates, most beta-cells were located in a central position but frequently showed cytoplasmic extensions between outlying non-beta-cells.

Conclusions: Human islets have a unique architecture allowing all endocrine cells to be adjacent to blood vessels and favouring heterologous contacts between beta- and alpha-cells, while permitting homologous contacts between beta-cells.
Islets of Langerhans are micro-organs located in the pancreas and composed by at least 4 types of endocrine cells. The alpha- and beta-cells are the most abundant and also the most important in that they secrete hormones (glucagon and insulin, respectively) crucial for glucose homeostasis. The prevailing description of islet cell composition and structure comes from studies performed in rats and mice. It is generally accepted that endocrine cells are not randomly distributed into islets. In most rodents, beta-cells compose the core of the islets and the non-beta-cells, including alpha-, delta- and PP-cells, form the mantle region. This unique architecture seems to have some functional implications (1). For instance, in several murine models in which insulin secretion is decreased, normal organisation of islet cells was found to be perturbed (2) so that beta-cells were intermingled with non-beta-cells. In addition, in vitro experiments showed that homologous contacts between rat beta-cells improved their function, as heterologous contacts between beta- and non-beta-cells had no effect (3). This observation suggests that a core-mantle segregation of islet cells is useful in favouring homologous contacts between beta-cells which in turn improves insulin secretion. The characteristic islet architecture may also serve to facilitate interactions between the different islet hormones via interstitial or vascular routes (4; 5).

The sparse works on the structure of human islets do not provide a clear description of their cellular organisation. There is a consensus on the different endocrine cell types, which do not differ significantly between rodent and human islets, and on the proportion of islet beta-cells that is lower in humans compared to rodents (6-8). Controversies persist about the topographical arrangement of endocrine cells within human islets. Even though human islets are sometimes still presented with a simple core-mantle architecture similar to that of rodent islets, many reports described decades ago human islets with a different cell organisation (1; 9-11). Pioneer works from Orci and Unger (1) depicted human islets with alpha- and delta-cells located in the mantle and grouped against capillary walls within the core of beta-cells. It has been also proposed that human islets were subdivided into lobules or subunits comprising clusters of beta-cells surrounded by alpha-cells (9; 11) and that these lobules or subunits were separated by vascularised connective tissue and non-beta-cells (9). Grube et al. (10) proposed a different organisation where endocrine cells were organised in a ribbon like manner rather than in separated subunits. In their model beta-cells are located in the islet core and alpha-cells are arranged at the periphery and along intraislet capillaries. These views were challenged by more recent publications claiming that endocrine cell types were dispersed throughout the human islets (8; 12). A summary of this controversy has been skilfully reviewed by Bonner-Weir and O’Brien, who furthermore described human islets as complex cell arrangements with different profiles including cloverleaf pattern (6).

The purpose of this work was to bring some clarifications to this controversy by describing our observations on the distribution of alpha- and beta-cells in human islets.

**RESEARCH DESIGN AND METHODS**

**Pancreas procurement.** Human pancreata were harvested from adult heart-beating, brain-dead donors and designed to be processed for islet isolation and transplantation. For different motives (prolonged ischemia time, suspicion of tumours, etc) some pancreata were not processed for islet isolation and small specimens were taken for histology.
Specimens from 21 donors were used for these analyses. Sixteen specimens were from non diabetic donors with a mean age of 40.1 ± 16.2 years (range: 15-69 years) and a mean body mass index (BMI) of 25.2 ± 2.9 (range: 20-29). Five specimens were from 5 type 2 diabetic donors with a mean age of 57.8 ± 9.1 years (range: 42-65 years) and a BMI of 32.3 ± 10.0 (range: 26-50). Glycemia at the time of hospital admission was 8.9 ± 3.8 mmol/l (mean ± SD, n=12) for non diabetic donors and 13.9 ± 5.3 mmol/l (mean ± SD, n=5) for type 2 diabetic donors. Specimens from non diabetic donors were obtained from the head (n=4), the body (n=2), the tail (n=5) and unspecified regions (9) of the pancreas (for 4 pancreata, specimens from both the head and the tail were available). Specimens from type 2 diabetic donors were obtained from the head (1), the body (2) and the tail (2) of the pancreas. Histopathological analysis of the diabetic specimens revealed no apparent alteration of the islets. However, by double immunofluorescence for insulin and amylin (using a rabbit anti-amylin antibody purchased from Progen Biotechnik GmbH, Heidelberg, Germany), several beta-cells were found to be positive for amylin, in all 5 specimens. In one specimen, extracellular staining for amylin was also observed. Mouse pancreata were harvested from C57/BL6 mice (Janvier Laboratories, Le Genest-Saint-Isle, France). To this end, mice were first anesthetised and then exsanguinated by aortic sectioning. Pancreata were rinsed in PBS before processing for histology.

**Islet and islet cell isolations.** Human islet isolations were performed as previously described according to the Ricordi method with local adaptations (13; 14). The use of human islets for research was approved by our local institutional ethical committee. After purification, islets were cultured overnight at 37°C, and at 25°C thereafter, in CMRL medium containing 5.6 mmol/l glucose and supplemented with penicillin, streptomycin, glutamine, HEPES and 10 % fetal calf serum (hereafter referred to as complete CMRL). Islets used for morphological analysis were cultured for a total of 36-48 h. For islet cell dispersion, cultured islets were rinsed in PBS and incubated in Accutase (Sigma, St Louis, MO, USA) for about 9 min with occasional pipetting. Single cell suspensions (~90 % single cells) were rinsed with complete CMRL and aliquots of 10^5 cells were incubated for 24 h at 37°C in non adherent 60- mm-diameter Petri dishes containing 6 ml complete CMRL. Cells were harvested and attached within Cunningham chambers (15) for immunofluorescence assessment.

**Historical sections of pancreata and isolated islets.** Pancreas samples were incubated for 24 h in (methanol free) 10 % formalin, dehydrated and embedded in paraffin. Isolated islets were incubated 24 h in methanol free 10 % formalin, then deposited at the bottom of flat-bottomed tubes, embedded in agar to immobilize them, dehydrated and finally embedded in paraffin. All pancreata and islet samples were sectioned at 5 μm. For one pancreas sample, 80 consecutive sections were performed.

**Attachment of islet cells within Cunningham chambers.** Islet cells cultured 24 h in complete CMRL were harvested and injected into poly-L-lysine-coated Cunningham chambers (15). After 1 h at 37°C, chambers were rinsed to remove unattached cells and filled with methanol free 10 % formalin. After 20 min incubation at room temperature, they were rinsed with PBS and stored at 4°C before being used for immunofluorescence.

**Immunofluorescence.** Before immunofluorescence, sections were deparaffinized and rehydrated with a series of alcohol solutions of decreasing concentration. Sections and cells attached to Cunningham chambers were then washed with phosphate buffer saline (PBS).—Antibody dilutions and rinsing steps were performed in PBS.
Incubations were done at room temperature, except as indicated below. For double staining (insulin + glucagon, insulin + somatostatin or insulin + PP), slides were treated 20 min with 0.5% Triton X100 and 20 min with 0.1% BSA, at room temperature. Slides were then incubated 1 h with a mouse anti-glucagon antibody (1:3000; Sigma), a rabbit anti-somatostatin antibody (1:300; Dako, Carpinteria, CA, USA) or a rabbit anti-PP antibody (1:300; Dako). After rinsing, slides were incubated 1 h with either an Alexa 488-conjugated anti-mouse antibody (1:500; Invitrogen, Basel, Switzerland) or a FITC-conjugated anti-rabbit antibody (1:400; Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Then, slides were rinsed and incubated 1 h with a guinea-pig anti-insulin antibody (1:1000; Dako) and successively 1 h with a Rhodamine-conjugated anti-guinea-pig antibody (1:100, Jackson ImmunoResearch Laboratories). For triple staining (insulin, glucagon and CD34), deparaffinized sections were treated 10 min at 37°C with a 0.1% trypsin solution, 20 min with 0.5% Triton X100 and 20 min with 0.1% BSA, at room temperature, and incubated successively 1 h with a mouse anti-CD34 antibody (1:50; Serotec, Oxford, UK), 1 h with a 488-Alexa conjugated anti mouse antibody (1:500; Invitrogen), 1 h with rabbit anti-glucagon (1:50; Dako) and guinea-pig anti-insulin (1:1000; Dako) antibodies and 1 h with a Rhodamine-conjugated (1:750) anti-rabbit and AMCA-conjugated anti-guinea-pig antibodies (1:150; Jackson ImmunoResearch Laboratories).

**Morphological analysis and quantifications.** Microscopic sections and islet cells were analysed with an Axioskop microscope (Zeiss, Feldbach, Germany) equipped with UV illumination and filters for blue, red and green fluorescences. Images were captured with an Axiocam color CCD camera (Zeiss) and recorded on computer through the AxioVision™ software (Zeiss). Isolated cells were also analysed by confocal microscopy (Zeiss LSM510 Meta) configured for simultaneous analysis of red and green fluorescences. Quantifications on isolated cells were performed by microscope observation. Quantifications on islet and pancreas sections were performed on digital images. Analysis of red and green fluorescence on digital images was performed using the offline MetaMorph imaging software for microscopy (Universal Imaging Corporation, West Chester, PA). This software was programmed to automatically quantify red and green stained areas within defined regions of interest. Data were expressed as mean ± SEM of n different experiments or islets. Differences between means were assessed either by the Student’s t-test and when required by one-way ANOVA. When ANOVA was applied, Scheffé’s least-significant difference post-hoc analysis was used to identify significant differences.

**RESULTS**

When pancreas sections were double stained for insulin and glucagon, apparently small islets (40-60 μm-diameter) showed a segregated cell type distribution with alpha-cells localized in the mantle and beta-cells in the core of the islets (Fig. 1A and G). In apparently bigger islets (Fig. 1A-C), alpha-cells were found in the mantle as in 40-60 μm-diameter islets, and also along simple or ramified “empty” areas (Fig 1A-C) present inside these islets. Islets with apparent diameters of 50 to 100 μm generally displayed one simple empty area (Fig. 1A), whereas islets with apparent diameters over 100 μm displayed multiple and/or ramified empty areas (Fig 1A-C). Labelling for CD34 revealed that vessels surrounded the periphery of islets and localized inside these empty areas, hereafter referred to vascular channels. (Fig. 1H and I). Careful examination showed that alpha-cells were always lining the vascular channels and the mantle region of the
islets, and juxtaposed to endothelial cells (Fig.1G-I). Interestingly, no CD34 staining was observed inside the beta-cell bulk, suggesting that vessels did not penetrate the beta-cell bulk. Similar observation was made for 40-60 μm-diameter islets. Since in this analysis apparently small islets could be tangential sections of bigger islets, we also examined serial sections permitting to follow several islets (n=8) through their whole thickness. Thus, after a triple immunofluorescence for insulin, glucagon and CD34, we confirmed that no vascular channel penetrated the core of 40-60 μm-diameter islets or the clusters of beta-cells in bigger islets.

When double staining was performed for insulin and somatostatin or insulin and PP, we observed that delta- and PP-cells had roughly the same topographical position as alpha-cells (not shown).

To confirm segregation of alpha- and beta-cells, areas labelled for glucagon (green=a) and insulin (red=b) were quantified in three different subregions of each islet profile. These subregions were 1) the mantle defined as a region of 20 μm deep that follows the external perimeter of islets, 2) the core defined as the total islet area minus the 20 μm-mantle area and 3) the vessel area defined as a region of 20 μm deep that follows the contour of vessels (Fig. 2A). The results expressed as b/(a+b) ratios were 0.71 for the core and 0.50 for the mantle and the vessel area of islets (Fig. 2B), showing that beta-cells preferentially localized at the core compared to mantle and vessel area. Considering that single cell section areas are roughly similar between alpha- and beta-cells, one can extrapolate that the core contains 3 times more beta-cells than alpha-cells, whereas the mantle and the region around vascular channels contain roughly the same number of alpha- and beta-cells. When subregions were selected with a 10 μm instead of a 20 μm rim, the differences in ratios between subregions were amplified (not shown), correlating with the observation that the alpha-cells were lining the islet mantle and vessels, as a single layer (~10 μm). The mean apparent diameter of all islets analysed here was 154 ± 4 μm (mean ± SEM of 255 islets from 21 different pancreata).

When pancreatic islets from type 2 diabetic donors were similarly analysed, an equivalent pattern of islet cell distribution was observed (Fig. 2C). However, the range of values was lower in diabetic compared to control islets. This last observation is in agreement with published works showing a decrease of beta-cell mass in type 2 diabetes (16-19).

We then studied the profile of b/(a+b) values in cultured isolated islets. After enzymatic digestion, the islet vasculature is damaged and cell arrangement is expected to be perturbed after culture. Section analysis of these islets revealed that few structures resembling vascular channels were remaining. Total profile area of these structures was 8 times lower in isolated islets compared to profile area of vascular channels in islets in situ. Morphometric analysis indicated similar b/(a+b) ratios between the core and mantle of cultured isolated islets (Fig. 2D).

When analysis of control pancreatic islets was performed according to their apparent diameter, b/(a+b) ratios in the mantle remained constant (Fig. 3A), as ratios in the core gradually decreased with the increasing islet apparent diameter (Fig. 3B). This observation confirmed that apparently small islets (50-100 μm-diameter) had a core-mantle organisation close to that of rodent islets (Fig. 2D), and that apparently bigger islets had elevated numbers of non-beta-cells in their core.

To gain an insight into the islet cell organisation, we performed consecutive serial sections through pancreas specimens and a three-dimensional analysis of islets (Fig. 4). It appeared evident that islet cells were not organised into discrete core-mantle subunits.
as previously described (9; 11). Rather, islet cells were organised into a continuous structure, like a folded epithelial plate (board) that span in a 3D network throughout the whole islet. The epithelial plate, whose thickness did not vary much, had a triple-laminated section with a central part of beta-cells lined at both sides with alpha- and other islet cells. Moreover, vessels were juxtaposed at both sides of the epithelial plate structure, invaginating in and following its folds. In 54 islets from 5 different pancreata, we measured the distance between vascular channels, equivalent to the thickness of the trilaminar plate. The value observed of 42 ±13 m (mean ± SD) roughly fits the diameter of the smallest islets.

Alpha-cells, due to their position within the epithelial plate, are exclusively aligned along vessels. Beta cells were also found aligned directly along vessels in alternation with alpha cells. Where alpha cells were the most numerous, they formed like a fence between endothelial cells and beta cells. In this case, beta cells appeared to form a second layer of cells over the first layer of alpha-cells. However a careful examination of these cells revealed that they develop extensions that infiltrated between alpha-cells and progressed until the surface of endothelial cells (Fig 5A-F). Confocal microscopic analysis showed that narrow strips of insulin staining between alpha cells corresponded to true cytoplasmic extensions and not to insulin released into extracellular compartment. Indeed, confocal analysis revealed that insulin staining between alpha-cells had a granular pattern similar to that observed elsewhere inside beta-cells, and colocalized with actin staining (see Supplemental Figure in the online appendix which is available at http://diabetes.diabetesjournals.org) As a consequence of this cell organisation, the heterologous contacts between alpha- and beta-cells around vessels are most numerous than homologous contacts between beta- or alpha-cells (Fig. 5G). When association between alpha- and beta-cells was assessed in cultured isolated islet cells, we observed many alpha-cells wrapped by beta-cells and rarely the contrary (Fig. 6). Of all alpha-cells contacting a beta-cell, 38 ± 8 % (mean ± SEM of 3 experiments) were round and had a perimeter almost completely wrapped by a beta-cell. This result revealed a unique plasticity of beta-cells that were able to spread around alpha-cells and suggested that this characteristic was intrinsic to beta-cells and not dictated by some islet coercions, such as extracellular matrix or islet vasculature.

The specimens used to analyze the pancreatic islet architecture were from different regions of the pancreas, including the head, the body and the tail. We did not observed obvious variations in islet structures according to the regional origin of the pancreas specimens. To further investigate this point, we compared head and tail specimens from the same pancreas (n=4). Islets with the trilaminar plate structure were observed in both head and tail regions of all pancreata.

**DISCUSSION**

In this morphological analysis, we show that islet cells are organised into a trilaminar plate comprising one layer of beta-cells sandwiched between two alpha-cell-enriched layers. This structure has a folded pattern and vessels circulate along both of its sides. These observations are schematized in Figure 7. No evident vascular channel penetrates the central layer of beta-cells inside the trilaminar plate, but many vascular channel profiles are found adjacent to the alpha-cell-enriched layers. Absence of CD34 staining within the beta-cell layer strongly suggest absence of endothelial cells. If some endothelial cells unlabeled for CD34 were nevertheless present, they ought to form tiny and collapsed vessels since no structures resembling typical vessels were observed. There is no doubt that most, if not all, alpha-cells are in direct
contact with vasculature. With regard to beta-cells, many are intercalated between alpha-cells and clearly in contact with blood vessels. Other beta-cells set on a layer of alpha-cells and display cytoplasmic extensions that run between alpha cells to reach the vessel surface as well. Other beta-cells are more distant from vessels and doubts persist as to their ability to reach the vasculature. A more meticulous 3D analysis could be able to answer this question.

No endothelial cells were observed in the core of 40-60 μm-diameter islets presenting a core-mantle organisation, suggesting that vessels do not penetrate these islets. But here again, beta-cell extensions intercalated between peripheral alpha-cells were observed and it is possible that most beta-cells reach the surrounding vasculature by this way.

Our description of human islet cell organisation is innovative but does not contradict the different views reported so far about the human islet architecture (6; 8; 9; 11; 12), including the diametrically opposed opinions describing islets with either a segregated or intermingled cell types. Indeed, a clear segregation between alpha- and beta-cells was observed and this was particularly true in small human islets where alpha-cells surrounded a core of beta-cells. In larger human islets, a kind of cell segregation was also observed since alpha-cells were mostly confined at the periphery of the trilaminar plate. From another point of view, human islet cells can be considered as intermingled, since we clearly showed that intercellular contacts between alpha- and beta-cells predominate. This is certainly a consequence of the trilaminar organisation and cytoplasmic extensions of beta-cells that intercalate between alpha-cells. This is not the case in rodent islets where the higher degree of cell segregation clearly favours beta- and alpha-cell homologous contacts.

The potential physiological significance of heterologous contacts between alpha- and beta-cells is suggested by works showing that glucagon positively affected insulin release (20; 21). In this respect, we recently demonstrated that insulin secretion from individual beta-cells was increased when they were in contact with alpha-cells (22). These results combined with those showing that heterologous contacts predominate in human islets suggest that glucagon is a more potent regulator of insulin secretion in human than in rodent islets.

Heterologous interactions between beta- and alpha-cells in human islets are not only frequent but also unusual, since beta-cells most often wrap the neighbouring alpha-cells. This behaviour of beta-cells towards alpha-cells was also observed in vitro in cultured isolated islet cells, demonstrating that it is innate to cells and not commanded by some islet constraints. The molecular mechanisms that account for this phenomenon have not yet been studied. Most likely, cytoskeleton and adhesion molecules expressed by islet cells may play a role. A physiological relevance of this unique plasticity of beta-cells is possible, especially considering that glucose-induced insulin secretion in vitro is improved in spreading beta-cells compared to round-shaped beta-cells (23) and that cytoskeleton plays a role in insulin secretion (24). Cytoskeleton organisation could be modified in beta-cells wrapping alpha-cells and spreading beta-cells. It remains to be demonstrated whether this modification has a direct impact on insulin secretion.

Islets within pancreata of type 2 diabetic donors have roughly the same structure as islets from non-diabetic donors. Indeed, we have found that the associations between beta- and alpha-cells were roughly similar in type 2 diabetic and non-diabetic islets, suggesting that the beta-cell dysfunction observed in type 2 diabetes is not related to a clear abnormality of islet cell organisation. Certainly, more studies are required to understand if a more subtle defect in cell organisation is involved. Our quantitative
analysis demonstrated that beta-cell mass relative to alpha-cell mass was substantially decreased in type 2 diabetic islets. These results are in agreement with some previous reports showing a decrease of the absolute beta-cell mass or of the volume of islets relative to the volume of pancreas in type 2 diabetic subjects (16-19).

We have shown that the unique organisation of islet cells observed within human pancreata is not maintained in cultured isolated islets. This is not surprising considering the insults sustained by islets during the isolation procedure, including disruption of vascularisation, enzymatic digestion of extracellular matrix and mechanical shaking of the tissues. Islet morphology could be also affected by culture. Indeed intercellular contacts are under the control of molecular events influenced by environmental factors. Finally, the handling, fixation and paraffin embedding of islets may also affect the cellular structure of the islets. It would be interesting to investigate if islet architecture is maintained in freshly isolated islets and what is the real effect of the culture on islet morphology. Many components of the medium could affect islet morphology in vitro. Among these components, there are the insulin secretagogues, such as glucose, that have been shown to affect expression of adhesion molecules, including E-cadherin and integrins, in islet cells (23; 25). In rodents, dissociated islet cells readily reaggregate in culture and after few days are able to form pseudoislets with a core-mantle organisation similar to that of native islets. This indicates that in rodents, information that decides of the islet architecture is foremost provided by islet cells themselves (26; 27). Our observations suggest that islet cells by themselves are not responsible for the unique cellular organisation of human islets. In humans, the more complex organisation of islets could involve more complex mechanisms. The role of vessels and extracellular matrix components in the maintenance of human islet architecture remains to be investigated. Furthermore, it will be particularly interesting to determine whether islet morphology is restored after transplantation and whether the unique arrangement between alpha- and beta-cells is critical for the function of transplanted islets.

ACKNOWLEDGEMENTS
This work was supported by grants from the Swiss National Science Foundation (3200BO-120376). Human islets were obtained thanks to a grant from the Juvenile Diabetes Research Foundation (31-2008-416). We thank Corinne Sinigaglia and David Matthey-Doret for their excellent technical assistance.
REFERENCES

**Figure legends**

*Fig. 1. Organisation of alpha- and beta-cells in human pancreatic islets.* Sections of human pancreata with islets of different sizes were either double-labelled for insulin (red) and glucagon (green) (A-C) or triple-labelled for insulin (blue), glucagon (red) and CD34 (green) (G-I). D-F and J-L: Outlines of endocrine tissue labelled for insulin and glucagon were drawn. Except for the 40-60 μm-diameter islets, all islets displayed one or several unstained empty areas (vascular channels) at their core. Most glucagon-expressing cells were located around vascular channels and at the mantle of islets, independently of their size. Insulin-expressing cells seemed clustered into discrete ovoid areas surrounded by alpha-cells. In triple labelled sections (G-I), vascular channels displayed staining for CD34 indicating that they contained vessels. Islets shown are representative of at least 200 islets observed on sections from 16 different pancreata. Scale bars, 50 μm.

*Fig. 2. Distribution of alpha- and beta-cells according to different defined islet subregions.* A: Pancreatic islet labelled by immunofluorescence for insulin (red) and glucagon (green); rims (white lines) delimiting mantle and core subregions and one vascular channel area are shown; scale bar, 10 μm. After immunofluorescence, areas labelled for insulin (b) and glucagon (a) were measured and results expressed as b/(a+b) ratio. This ratio was calculated for areas measured in the different subregions (mantle, core and vessels) and in the whole islets (whole). Analyses were performed on histological sections from non diabetic human (B), type 2 diabetic human (C) and control mouse (E) pancreata and cultured isolated human islets (D). Columns are means ± SEM. B: n=193 islets from 16 pancreata, C: n= 54 islets from 5 pancreata, D: n=20 islets from 2 pancreas, E=50 islets from 1 pancreas. ND = not determined.

*Fig. 3. Distribution of alpha- and beta-cells according to islet apparent size.* Islets from human pancreas sections analysed in Fig. 2 are shown here according to their apparent diameter. After
immunofluorescence, insulin and glucagon labelled areas were measured and results expressed as $b/(a+b)$ ratio, where $a$ and $b$ were representing glucagon and insulin labelled areas, respectively. 

**A:** $b/(a+b)$ ratio calculated for areas measured in the 20 μm mantle part of islets. 

**B:** $b/(a+b)$ ratio calculated for areas measured in the core of the islets. Columns are means ± SEM. 

n=39 for islets with 50-100 μm diameter, 60 for islets with 100-150 μm diameter, 58 for islets with 150-200 μm diameter, 24 for islets with 200-250 μm diameter, 12 for islets with 250-300 μm diameter.

**Fig. 4.** Three-dimensional (3D) analysis of human pancreatic islets. Consecutive sections through an entire pancreatic islet labelled for insulin (red) and glucagon (green). Images show that insulin (red) and glucagon (green) stained cells are organised into continuous 3D networks that span throughout the entire islet. Sections at both ends show islet profiles with an apparent similar core-mantle structure to that of 40-60 μm-diameter islets. These consecutive images also reveal that vascular channels were in fact continuous ramified structures that were connected in places with the surrounding islet tissue (arrowheads). Image series is representative of 15 different pancreatic islets analyzed from one pancreas. Scale bar, 50 μm.

**Fig. 5.** Unique association between alpha- and beta-cells in pancreatic islets. Pancreatic islets labelled for glucagon (green) and insulin (red) are shown at low magnification (A and D). Boxed areas are shown at higher magnification in B and E. Alpha- and beta-cells lining vascular channels in B are depicted in C. Alpha- and beta-cells lining the islet mantle in E are depicted in F. Arrow heads point cytoplasmic extensions of beta-cells that span over alpha-cells. Scale bars, 10 μm in A and D, and 5 μm in B and E. Scale bars, 10 μm. Heterologous contacts between beta- and alpha-cells (beta-alpha) and homologous contacts between alpha-cells (alpha-alpha) and beta-cells (beta-beta) around empty areas were scored. Their relative frequencies are shown in G. Columns are means ± SEM, n = 52 islets from 10 pancreata.

**Fig. 6.** Unique association between alpha- and beta-cells in cultured islet cells. Human islet cells were isolated and cultured for 24 h. After a double immunofluorescence for insulin (red) and glucagon (green), islet cells were analyzed by confocal microscopy. A-C: Images showing a cell pair composed of one alpha-cell (A) surrounded by one beta-cell (B); the merged image is shown in C. The cell pair showed here is representative of cell pairs observed in different human cell preparations from at least 10 different pancreata. D: One series of consecutive merged images of a cell pair composed of one alpha-cell (green) surrounded by a beta-cell (red). Scale bars, 10 μm. E: All heterologous contacts between alpha- and beta-cells were scored according to their type of association: a beta-cell wrapping an alpha-cell (beta wrapping alpha), neutral apposition between alpha- and beta-cells (alpha-beta) and an alpha-cell wrapping a beta-cell (alpha wrapping beta). Results are shown as relative frequencies and columns are means ± SEM of 5 islet cell preparations from 5 different pancreata. From all heterogeneous contacts between alpha- and beta-cells, the percentage of alpha cells whose profile was round and perimeter almost completely wrapped by a beta-cell as in D was 38 ± 8 (mean ± SEM of 3 experiments).

**Fig. 7.** Model of endocrine cell and vessel organisation in human islets. A: Alpha-cells (green) and beta-cells (red) are organised into a thick folded plate lined at both sides with vessels (blue). Alpha-cells are mostly at the periphery of the plate and in close contact with vessels. Beta-cells occupy a more central part of the plate and most of them develop cytoplasmic extension that run between alpha-cells and reach the surface of vessels. B: The plate with adjacent vessels is folded so that it forms an islet.
cell arrangement in human islets of Langerhans

Figure 1

A

B

C

D

E

F

G

H

I

J

K

L
Figure 2

A figure showing a histological section of human islets of Langerhans with a bar graph illustrating the b/(a+b) ratio for different regions: whole, mantle, core, and vessels. The graph compares these ratios across different categories: whole, mantle, core, and vessels.
Figure 3

A

B

islet diameter (μm)

islet diameter (μm)
Figure 4

Cell arrangement in human islets of Langerhans
Figure 5

A
B
C
D
E
F

G

Frequency (%)

beta-alpha  alpha-alpha  beta-beta
Figure 6

cell arrangement in human islets of Langerhans
cell arrangement in human islets of Langerhans

Figure 7