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ARTICLE

Blood vessels of human islets of Langerhans are surrounded by a double basement membrane

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Abstract
Aims/hypothesis Based on mouse study findings, pancreatic islet cells are supposed to lack basement membrane (BM) and interact directly with vascular endothelial BM. Until now, the BM composition of human islets has remained elusive.
Methods Immunohistochemistry with specific monoclonal and polyclonal antibodies as well as electron microscopy were used to study BM organisation and composition in human adult islets. Isolated islet cells and function-blocking monoclonal antibodies and recombinant soluble Lutheran peptide were further used to study islet cell adhesion to laminin (Lm)-511. Short-term cultures of islets were used to study Lutheran and integrin distribution.
Results Immunohistochemistry revealed a unique organisation for human Lm-511/521 as a peri-islet BM, which co-invaginated into islets with vessels, forming an outer endocrine BM of the intra-islet vascular channels, and was distinct from the vascular BM that additionally contained Lm-411/421. These findings were verified by electron microscopy. Lutheran glycoprotein, a receptor for the Lm α5 chain, was found prominently on endocrine cells, as identified by immunohistochemistry and RT-PCR,

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whereas α_3 and β_1 integrins were more diffusely distributed. High Lutheran content was also found on endocrine cell membranes in short-term culture of human islets. The adhesion of dispersed beta cells to Lm-511 was inhibited equally effectively by antibodies to integrin and α_3 and β_1 subunits, and by soluble Lutheran peptide.
Conclusions/interpretation The present results disclose a hitherto unrecognised BM organisation and adhesion mechanisms in human pancreatic islets as distinct from mouse islets.

Keywords Basement membrane · Laminin · Langerhans islet · Lutheran · Vessels

Abbreviations

B-CAM	basal cell adhesion molecule
BM	basement membrane
CLSM	confocal laser scanning microscopy
ECM	extracellular matrix
EHS	Englebreth–Holm–Swarm
Lm	laminin
Lu	Lutheran
MAb	monoclonal antibody
PECAM-1	platelet endothelial cell adhesion molecule-1

Introduction

Signals originating from the extracellular matrix (ECM are important for pancreatic islet development and beta cell function [1–3]. Laminin (Lm)-111 ($\alpha1\beta1\gamma1$ according to new Lm nomenclature [4]) plays an important role in pancreatic organogenesis [5] and differentiation of early pancreatic cells into insulin-positive beta cells [6, 7], but is not present in adult mouse [8] or human pancreas [9]. In contrast, collagen IV has been found to inhibit mouse pancreatic development, while other ECM molecules like fibronectin played no apparent role [6].

Laminins, together with type IV collagens, are major basement membrane (BM) glycoproteins. Both are able to self-assemble into independent networks connected by nidogen. Laminins form a family of trimeric glycoproteins composed of an α, β and γ chain. The Lm chains can combine into 15 different Lms, which are found in a cell- and tissue-specific manner [4, 10].

Numerous studies have suggested that BMs of most glandular organs contain Lm-332 and Lm-511, but both of these Lms are lacking in BMs of mouse pancreas, which only contain Lm-211 and Lm-411 [11, 12]. Both these studies and a recent study [13], the subject of which has been reviewed [14], suggest that the only BM found in the mouse islets is that surrounding the vascular endothelium. In addition, Nikolova et al. [13] showed an islet capsule containing BM proteins that are not produced by endothelial cells. Some findings have indicated that in the human pancreas, endocrine cells are separated from exocrine cells by reticulin fibres [15] and the endocrine–exocrine interface in different species variably appears to contain collagens I, IV, V and VI, as well as Lm [16–18]. Electron microscopy detected [19] a typical BM facing the endocrine cell at the endocrine–exocrine interface as a peri-islet BM, but the same study failed to detect BM inside human islets. Recent studies have provided evidence of the unique cytoarchitecture of human pancreatic islets when compared with that of rodents [20, 21]. However, systematic studies on the BM proteins and their receptors in the human pancreas and islets are entirely lacking.

A detailed understanding of the human islet BM structure may be important for the pathogenesis of type 1 and type 2 diabetes. We have now studied BM protein composition and were able to localise them and their receptors in detail in human islets of Langerhans. Our results reveal important species differences between mice and humans.

Methods

Tissue specimens We retrieved 12 adult pancreas tissue samples obtained during surgery due to pancreatic carcinoma from the archives of the Institute of Biomedicine/Anatomy. The specimens had been frozen in liquid nitrogen immediately after removal. Tissue sections were stained with haematoxylin–eosin to confirm their normal histology. Adult mouse pancreatic tissues were obtained from *Balb/c* mice (n=4) and were frozen in liquid nitrogen.

Immunofluorescence and confocal laser scanning microscopy Monoclonal antibodies (MAbs) were used for indirect immunofluorescence (Electronic supplementary material [ESM] Table 1) and bound primary antibodies visualised using AlexaFluor-488-labelled goat anti-mouse or anti-rat or anti-rabbit IgG (Invitrogen – Molecular Probes, Eugene, OR, USA). In double-labelling experiments the sections were first exposed to MAbs and their conjugates and after washing three times in phosphate-buffered saline, pH 7.4, exposed to polyclonal antiserum and AlexaFluor-594-labelled goat anti-rabbit (Invitrogen). The specimens were embedded in veronal glycerol buffer (1:1, vol/vol., pH 8.4) or for confocal laser scanning microscopy (CLSM) in 90% glycerol/10% (vol./vol.) veronal buffer.

The specimens were viewed with an Aristoplan (Leica, Wezlar, Germany) microscope or an AX70 Provis fluorescence microscope (Olympus, Tokyo, Japan). Images were acquired using a computer connected to a cooled digital camera mounted on the microscope. CLSM was carried out

using a Leica TCS SP2 system with argon and krypton excitation line at 488 and 568 nm. Image stacks were collected through the specimen using a standardised 120 nm z-sampling density. Selected image stacks (n=9) were subjected to deconvolution and restoration using a theoretical point spread function and iterative maximum likelihood estimation algorithm (Scientific Volume Imaging, Hilversum, the Netherlands).

Transmission electron microscopy Normal pancreatic tissue from three adult patients undergoing pancreatic resection due to carcinoma was collected and small samples were immediately immersed for 6 h in 2% (vol./vol.) glutaraldehyde in 0.1 mol/l sodium cacodylate buffer, pH 7.2, supplemented with 0.1 mol/l sucrose, and then post-fixed for 60 min in 1% (wt/vol.) osmium tetroxide in cacodylate buffer. The samples were then dehydrated in ethanol and embedded in Agar 100 (Agar Scientific, Stansted, Essex, UK). The ultra-thin sections were cut and contrasted with uranyl acetate and lead citrate and analysed in an H-7100 electron microscope (Hitachi, Tokyo, Japan). The islets were located by using sections stained with Toluidine Blue.

All human tissues were obtained and handled in accordance with the principles expressed in the Declaration of Helsinki and the study was approved by the local medical Ethics Committees.

Human islet isolation and short-term culture Human pancreas was processed and islets were isolated as described earlier [22] in the Central Laboratory of the Nordic Network for Clinical Islet Transplantation in Uppsala, Sweden. After Ficoll gradient purification, fractions rich in islets (60–85%) were collected and shipped on ice to Helsinki. Informed consent was obtained for cell tissue donors and institutional Ethics Committees in Uppsala and Helsinki approved all procedures. Upon arrival, the islets were washed once with Connaught Medical Research Laboratories (CMRL) 1066 medium (Invitrogen, Carlsbad, CA, USA) and seeded on non-adherent culture dishes (Barloword Scientific, Stone, UK) in the same medium supplemented with antibiotics and 10% FCS (PromoCell, Heidelberg, Germany). After suspension the islets, cultured for 2 to 3 days, were washed once with DMEM medium and seeded on to glass coverslips to allow attachment and partial monolayer formation. Non-adherent islets were removed on the following day and the adherent islets were allowed to form monolayers for 48 to 72 h. Finally, the coverslips were fixed in methanol at −20°C for immunoreactions.

Cell adhesion studies were performed with dissociated islet cells. For this purpose the islets were dissociated with trypsin/EDTA solution and filtered through a 70 μm nylon mesh (Becton Dickinson Labware, Franklin Lakes, NJ, USA) to remove residual cell clumps. Before the experiment, the single cell suspension was incubated for 30 min at 37°C in a medium containing 2% FCS.

Cell adhesion experiments Cell adhesion was studied in 48-well plates containing glass coverslips. Human Lm-511 was purified from culture medium of Jar chorioncarcinoma cells by immunoaffinity chromatography beads as described [23]. Coverslips were incubated with Lm-511 (10 μg/ml) at room temperature for 1 h and the coverslips were washed twice with PBS. The coverslips were then incubated for another hour with soluble recombinant Lutheran (Lu) (4 μg/ml) corresponding to the extracellular domain of Lu [24, 25]. Single cells were counted and 8,000 to 10,000 cells were seeded on precoated coverslips in DMEM medium containing 2 g/l BSA. For integrin inhibition experiments, the cells were preincubated with MAbs against integrin β_1 subunit (MAb 13) or α_3 subunit (PIB5; Chemicon, Temecula, CA, USA). After incubation for 90 min, the medium containing non-adhered cells was collected and the coverslips washed with PBS and fixed immediately for 15 min with 4% paraformaldehyde. The results are presented as the mean±SEM of six separate experiments, except for the integrin antibodies (n=3).

RNA isolation and RT- PCR for Lu/basal cell adhesion molecule Total RNA was isolated from two different human islet preparations (purity 60 and 65%) and cultured human corneal epithelial (HCE) cells [26] using an RNA isolation kit (NucleoSpin RNA II; Macherey-Nagel, Düren, Germany). After separate treatment with DNaseI ROQ1 RNase-free (Promega, Madison, WI, USA) and RNA clean-up with NucleoSpin RNA Clean-Up kit (Macherey-Nagel), 1 μg total RNA was reverse-transcribed by M-MLV-Reverse Transcript-ase (Promega) in 20 μl RT reaction primed by Oligo(dT)15 primer (Promega).

We used 25 μl PCR containing 2 μl of RT reaction as template, plus 2.5 μl 10× PCR buffer with 15 mmol/l $MgCl_2$ (Applied Biosystems, Foster City, CA, USA), 2 μl of dNuTPs mix (contains 2.5 mmol/l of each dG/dC/dT dNuTP), 4 μl of the mixture of forward and reverse primers (stock of 2 μmol/l each), 2 μl of 50% (vol./vol.) DMSO and 0.2 μl AmpliTaq Gold 5 U/μl (Applied Biosystems). Polymerase activation/denaturation was performed at 95°C for 7 min and continued with 33 cycles (95°C 20 s, 56°C 30 s, 72°C 30 s). The common forward primer sequence for both of the human Lu and basal cell adhesion molecule (B-CAM) was 5′-CTACTGCGTGAGACGCAAAG-3′. The specific reverse primer for Lu (B-CAM variant 1) was 5′-GGTCTGCTCTGGTTGCTCC-3′and for B-CAM (variant 2) 5′-GGGGATGGGGTTAAGCTATG-3′. The PCR fragments synthesised were 115 bp for Lu (B-CAM v1; NM_005581, pos. 1748–1862) and 109 bp for B-CAM (v2 NM_001013257, pos. 1748–1856). For internal control

we used housekeeping gene cyclophilin G primers: forward 5′-ACTCCCAGCCTGCTTCATAC-3′ and reverse 5′-TACGTCTGAAACGATCCCTTG-3′, fragment 126 bp (NM_004792, pos. 1161–1286). PCR (5 ml) was gel-electrophoresed in 2% agarose gel.

Results

Molecular components of the human islet basement membranes We first studied the overall distribution of Lms in the islets using an antiserum to Englebreth–Holm–Swarm (EHS)-Lm that binds to most Lm isoforms with the exception of Lm-332. A continuous immunoreactive line representing peri-islet BM and surrounding the whole islet was found (Fig. 1) and was distinct from the acinar BM of exocrine pancreas. Inside the islet, capillary BM (Fig. 1) was further surrounded by a second and distinct outer endocrine BM (Fig. 1).

The Lm chain composition of these two distinct BMs was studied using MAbs. Immunoreactivity for Lm α1 chain was not found (Fig. 2a). In contrast, Lm α2 chain showed a bright reactivity in BMs of exocrine acini, while the islet BMs lacked immunoreaction (Fig. 2b). MAb against Lm α3 chain did not show reactivity in the islet (not shown). Immunoreactivity for Lm α4 chain was bright in the islet blood vessel BMs (Fig. 2c), but was not detectable in the peri-islet BM or in the outer leaflet of the duplex BM around intra-islet vascular channels. Immunoreactivity for Lm α5 chain surrounded the islets and acini (Fig. 2d,h). Inside the islets, immunoreactivity for Lm α5 chain was found in duplex BMs around blood vessels, but only in regular single BMs in the exocrine pancreas. Immunoreactivity for Lm β1 chain was similarly distributed in the single-layered peri-islet, peri-acinar and parenchymal perivascular BMs and in the double-layered BMs around the intra-islet blood vessels (Fig. 2e). Immunoreactivity for Lm β2 chain was very intense, but confined to the intra-islet duplex BMs (Fig. 2f), being nearly negative in the exocrine pancreas with the exception of arterial walls feeding the intra-islet capillaries (not shown). In the high magnification picture (Fig. 2f) Lm β2 chain-reactive inner capillary BM (arrow) was surrounded by a separate Lm β2-immunoreactive outer BM. In the pancreatic tissue, Lm β3 and γ2 chain immunoreactivities were only found in BMs of larger ducts (not shown). Immunoreactivity for Lm γ1 chain was found in BM surrounding the islets and in the duplex BMs of the intra-islet vascular channels (Fig. 2g). Larger magnification, in the example shown for the Lm α5 chain (Fig. 2h), clearly shows the distinction of the peri-islet BM from that of the delicate acinar BMs in the exocrine pancreas. Figure 2h also clearly demonstrates a tangential section of an invaginating vessel (asterisk).

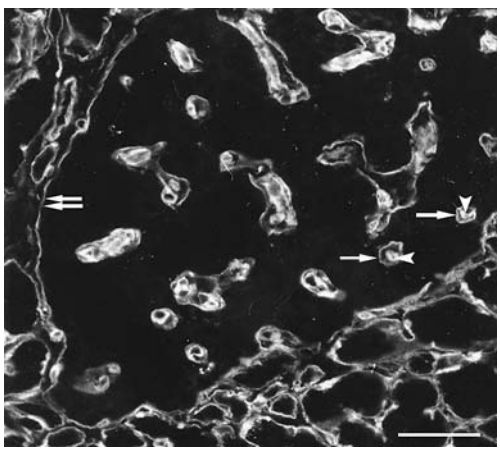

Fig. 1 Indirect immunofluorescence of human islet of Langerhans reacted with an antiserum to mouse-EHS Lm. A brightly immunofluorescent BM encircles the whole islet (double arrow) which is clearly distinct from the BMs of the acini of the exocrine pancreas. Intra-islet blood vessels are surrounded by a duplex BM consisting of an outer Lm-reactive parenchymal BM (arrows) that is distinct from the vessel and capillary BM (arrowheads). Bar, 50 μm

CLSM analysis of EHS-Lm immunoreactivity revealed a distinct reaction in endocrine parenchymal and capillary layers of the duplex BMs around the intra-islet vascular channels (Fig. 3a), whereas labelling with Lm α4 chain MAb (Fig. 3b) showed immunoreactivity only in BMs of the capillaries (Fig. 3c). Figure 3d–f shows high-resolution confocal views of an islet capillary immunoreactive for platelet endothelial cell adhesion molecule-1 (PECAM-1) (Fig. 3d) and EHS-Lm (Fig. 3e). The merged image (Fig. 3f) shows that PECAM-1 reactive endothelium underlies the inner endothelial BM and that the outer parenchymal BM is distinct from it.

Since the Lm network in the BMs is connected to type IV collagen by nidogen, we also studied the distribution of collagen IV (ESM Fig. 1) and nidogen (ESM Fig. 1b) immunoreactivities. MAbs against these BM proteins generated a strong immunoreaction both in the outer endocrine BM and the inner vascular endothelial BM, clearly exposing the double-layered nature of BM around blood vascular channels in the islets. ESM Figure 1 shows a vessel BM co-invaginating with the peri-islet BM.

The aforementioned results suggest the existence of a double-layered BM organisation around the vascular channels of human islets: the inner vascular leaflet of the duplex contains Lm-411/-421 and -511/-521, whereas the outer leaflet facing the parenchymal endocrine cells contains only Lm-511. In addition, Lm-521 was seen in the outer leaflet of the vascular channel facing the parenchymal endocrine cells. To further verify this unique double-layered BM organisation inside the islets, electron microscopy was

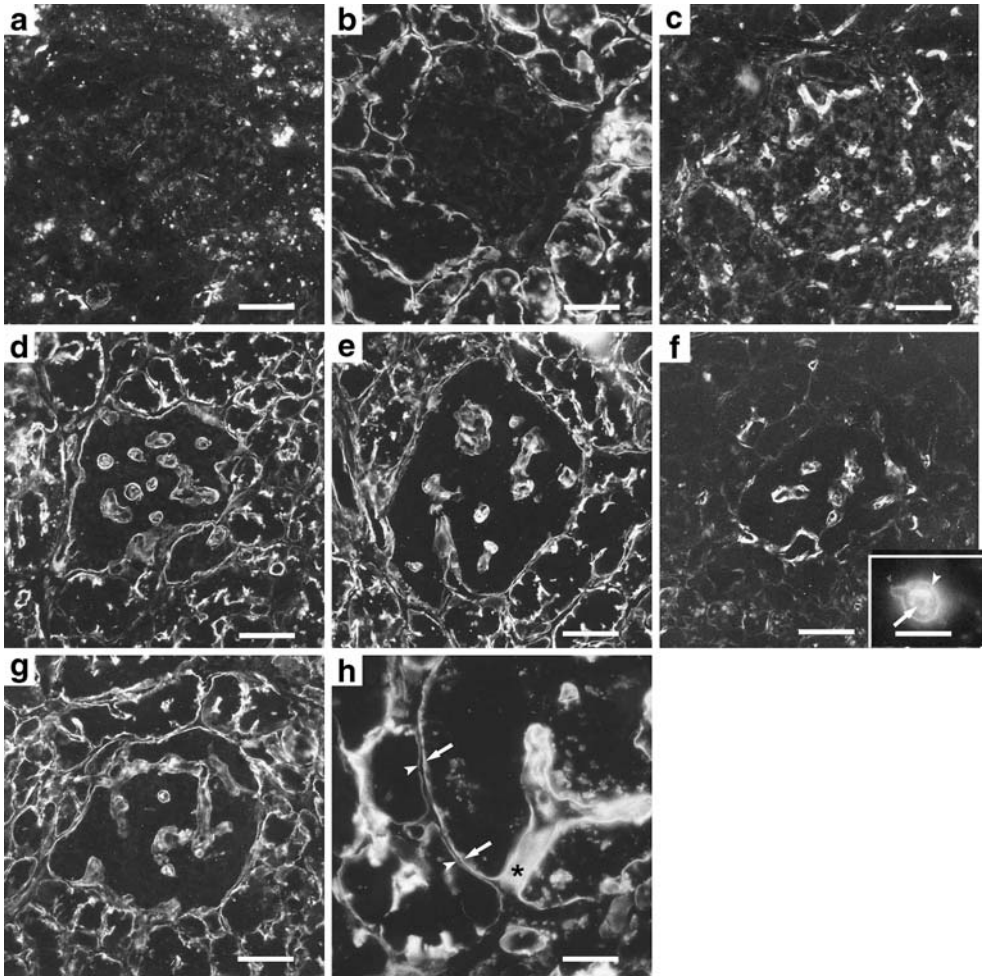

Fig. 2 Indirect immunofluorescence of human Langerhans islet with monoclonal antibodies against Lm α1 (**a**), α2 (**b**), α4 (**c**), α5 (**d, h**), β1 (**e**), β2 (**f**) and γ1 (**g**) chains. Immunoreactivity for Lm α1 and α2 chains is lacking in islet tissue although Lm α2 chain is prominent in the BM of the pancreatic acini (**b**). Immunoreactivity for Lm α4 chain (**c**) is only seen in the vascular BM, in islets and in exocrine pancreas. Lm α5 (**d**), β1 (**e**) and γ1 (**g**) immunoreactivities are seen as continuous lines around the islet and as duplex BMs around the intra-islet blood vessels separating the vascular endothelial and pancreatic endocrine cells supported by the inner and outer leaflet of the duplex BM, respectively. Lm β2 (**f**) chain is prominent in vascular BMs in the islets, whereas only traces of immunoreactivity are seen in the peri-islet BM and the exocrine pancreatic tissue. Higher magnification (insert) shows more clearly the outer BM facing the endocrine cells (arrowhead) and the inner vascular BM (arrow). **h** At a higher magnification immunoreactivity for Lm α5 chain is seen in BM surrounding the islet (arrows), which is clearly distinct from the BMs of the exocrine acini (arrowheads). Asterisk (*) (**h**), tangential section of a vessel invaginating into the islet tissue. Bars: **a–g** 50 μm; **f** insert, 10 μm; **h** 30 μm

used. Figure 4 shows a representative capillary in an islet. A distinct BM of the capillary endothelial cell can be seen, which is distinct from the BM of an adjacent endocrine cell (Fig. 4) and contiguous with the BM of a neighbouring endocrine cell (Fig. 4).

Laminin receptors in human islets of Langerhans Previous studies of Lm receptors in mouse and rat tissues have suggested that integrin α_3, α_6 and β_1 subunits are important for the development, organisation and function of rodent islets [2, 3, 18, 27–29]. Therefore, we studied the distribution of these and other integrin subunits as well as non-integrin Lm receptors in the human islet. Immunoreactivities for integrin β_1 (Fig. 5a) and integrin α_3 subunits (Fig. 5b) were rather diffuse and found on the vascular endothelia and endocrine walls of vascular channels (α_3), but also on endocrine cells. Integrin α_6 subunit in the islets was only found on vascular endothelia (Fig. 5c). No immunoreactivity for integrin α_2 or β_3 subunits was found, while immunoreactivity for α_v and β_5 subunits was diffuse (not shown). Some immunoreactivity for α-dystroglycan was found in a relatively typical punctate manner on the vascular endothelial cells (Fig. 5d); it coaligned with that of Lm α5 chain (Fig. 5e) as well as, on the outer margin of the islet, with peri-islet BM (Fig. 5d). In contrast, immunoreactivity for Lu glycoproteins was extremely strong, forming a continuous

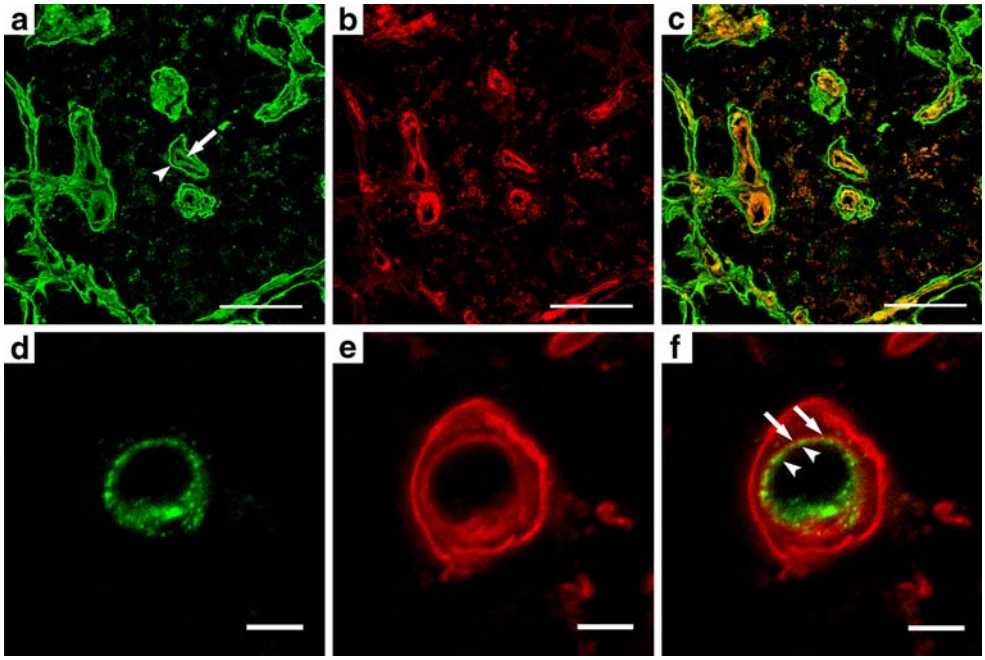

Fig. 3 CLSM of human Langerhans islets showing that the immunoreactivity for EHS Lm (**a**) forms BM surrounding the islet as distinct from that of the exocrine acini and a duplex BM around the blood vessel channels in the islet with a vascular inner BM sheet (arrow) distinguishable from the outer parenchymal endocrine BM (arrowhead). Immunostaining for Lm α4 chain (**b**) shows reactivity only in the capillary BM as shown in orange in the merged picture (**c**). A double immunoreaction for PECAM-1 (**d**) and EHS Lm (**e**) shows in nine confocal sections after deconvolution that the inner vessel BM, as seen in merged figure (**f**) (arrow), is lined inside by PECAM-1-reactive endothelium (arrowhead). Bars: **a–c** 30 μm; **d–f** 10 μm

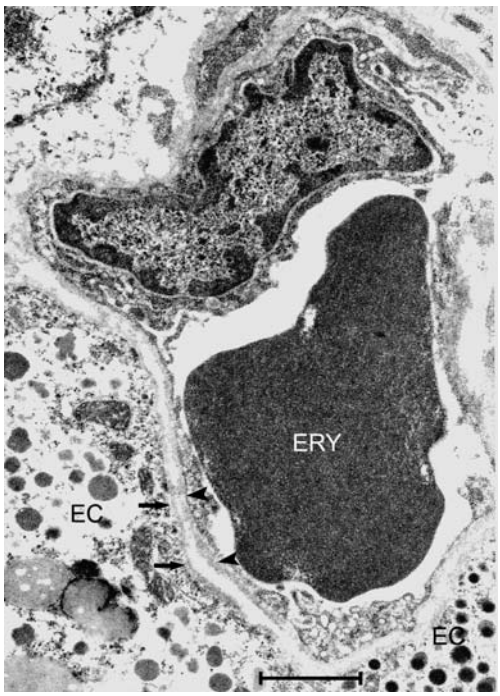

Fig. 4 Representative transmission electron microscopic (TEM) micrograph showing an erythrocyte (ERY) in a capillary blood vessel close to endocrine cells (EC). Arrowheads show the capillary BM, which is clearly distinct from BM (arrows) of the insulin- and glucagon-secreting cells. Original magnification: ×20,000. Bar, 1 μm

polarised band at the periphery of the islet and facing the peri-islet BM, and also facing the outer parenchymal endocrine BM of the vascular channels (Fig. 5f).

Exocrine acinar cells were also immunoreactive for Lu, but this reactivity was weaker. Double immunolabelling in CLSM using polyclonal antiserum revealed Lu (Fig. 5g) on the periphery of the islet, facing the peri-islet BM, and on the outer leaflet of the duplex BM surrounding the intra-islet vascular channels, with fainter Lu labelling of the vascular endothelial cells facing the inner leaflet of the duplex BM. Immunoreactivity for BM-confined Lm α5 chain (Fig. 5h) was located in close coalignment with that of Lu on the surfaces of the endocrine cells (Fig. 5i). Double immunostaining was also used to show that integrin α_6 subunit (ESM Fig. 2a) was colocalised with PECAM-1 (ESM Fig. 2b) in islets.

To show more precisely the localisation of Lu on islet endocrine cells, we used double immunofluorescence which showed a variable strong granular reactivity for chromogranin-A, a marker of neuroendocrine cells, in islet endocrine cells (ESM Fig. 3) and a strong polarised Lu-immunoreactivity (ESM Fig. 3b) on the periphery of chromogranin-A-positive cells.

Since on the basis of differential splicing, two distinct isoforms of Lu/B-CAM are known to exist [30], we used

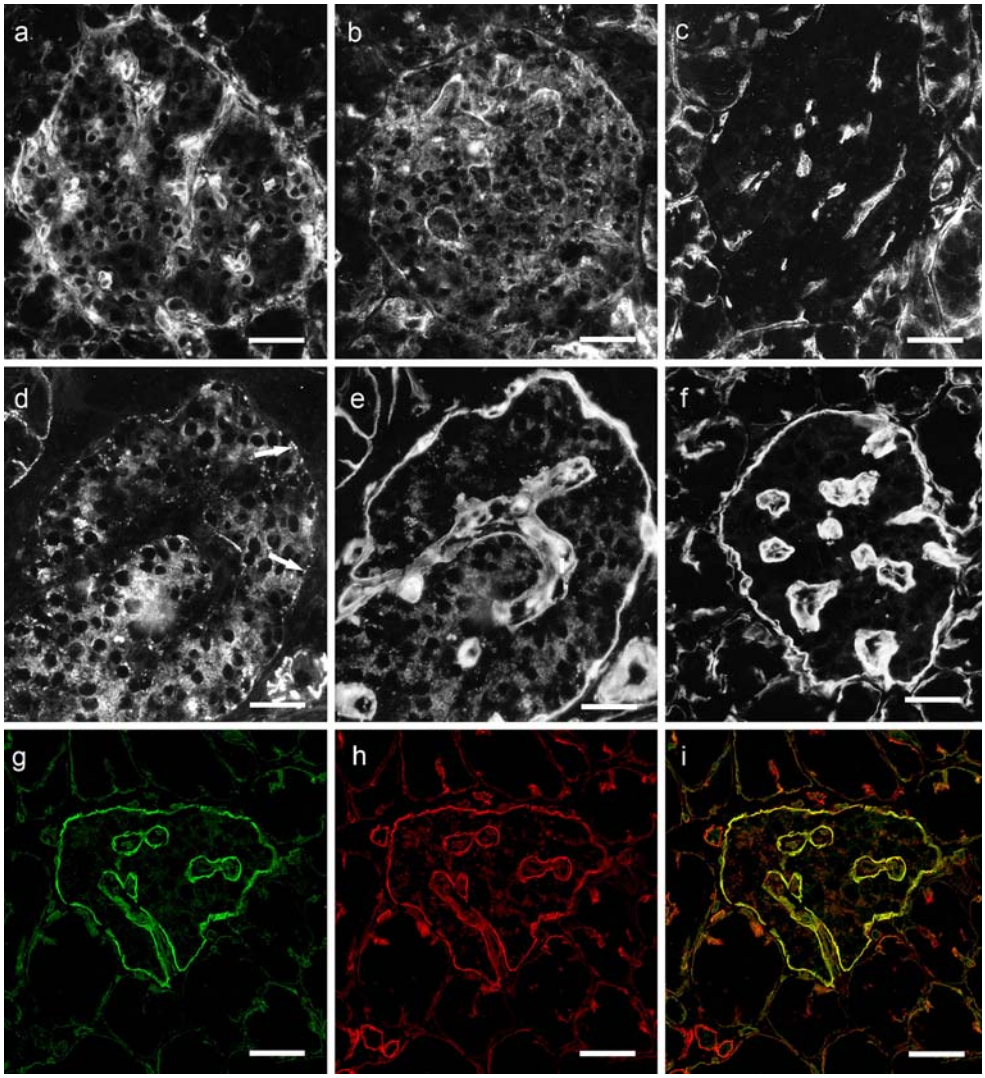

Fig. 5 Distribution of integrin and non-integrin Lm receptors in the islet. Immunoreactivity for integrin β_1 (**a**) chain is seen on endocrine islet cells and more clearly on vascular endothelia. Immunoreactivity for integrin α_3 subunit (**b**) is similar. Immunoreactivity for integrin α_6 subunit in the islets is only found on vascular endothelial cells (**c**). Typical punctate immunoreactivity for α-dystroglycan (arrows) (**d**) is scattered on endocrine and vascular endothelial cells partially coaligning with that of the much more strongly labelled Lm α5 chain (**e**). A very prominent immunoreactivity for Lu (**f**) is seen on the surface of these endocrine cells facing the exocrine pancreatic tissue in the periphery of the islet and in those facing the vascular compartment inside the islets. Immunolabelling with polyclonal antibodies against Lu (**g**) shows continuous and strong immunolabelling on the surface of the endocrine cells facing the BM that surrounds the islet and co-invaginates with the invading vessel being prominent on endocrine cells that cover vascular channels. Vascular endothelial cells show a weak immunoreactivity for Lu. **h** Double immunoreaction for Lm α5 chain was co-aligned with that of Lu (**g**) as shown also in orange in the merged picture (**i**). Bar, 50 μm

PCR with specific primers to identify these two mRNA variants in human islets. The 5′-primer is common to both mRNA forms, but the 3′-primers are designed specifically to distinguish the 3′-end of the Lu mRNA coding region from the mRNA of B-CAM not containing this region that codes the cytoplasmic src-homology 3 domain binding area and Ser/Thr phosphorylation sites. Positive PCR results were obtained only for transcripts of Lu isoform (variant 1), but not for the shorter B-CAM isoform (variant 2) (ESM Fig. 4).

The high content of Lu prompted us to study its distribution in short-term cultures of dissociated human islets. The islet cells adhered and part of them started to spread within the examination period, while the endocrine cells, detected by antisera against chromogranin-A or insulin, remained roundish. In such cultures, double immunoreaction for Lu and chromogranin-A (ESM Fig. 5a,b) showed that the brightly chromogranin-A-reactive cells had a distinct membrane-confined Lu-immunoreaction, while the adherent cells lacking chromogranin-A remained negative (ESM Fig. 5c). Immunoreactivity for integrin β_1 subunit was low but detectable in chromogranin-positive endocrine cells (ESM Fig. 5d), while the non-endocrine

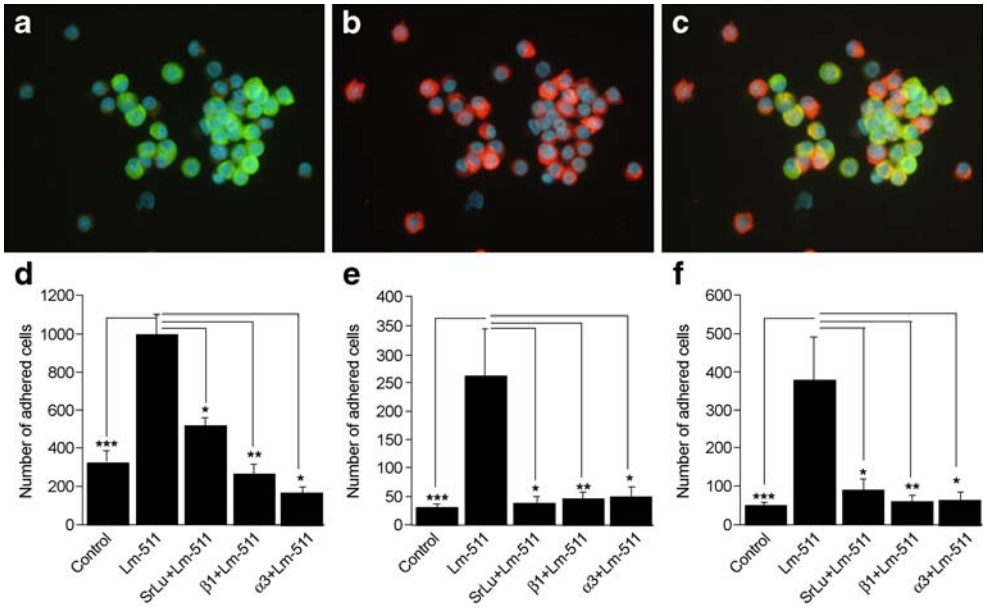

Fig. 6 Immunofluorescent images of cells double-stained for insulin (green) (**a**) or chromogranin A (red) (**b**) and both as overlay (**c**) after a 90 min adhesion experiment on Lm-511-coated substratum. Nuclear DAPI counterstaining showed that most cells were double-reactive for both antigens, while some cells (non-beta islet cells) only expressed chromogranin A. **d–f** Quantification of adhesion of 10 000 cells per well: total cells (**d**); insulin-reactive cells (**e**); chromogranin-A-reactive cells (**f**). The binding to Lm-511 was inhibited by soluble Lu (SrLu) and monoclonal antibodies against integrin β_1 or integrin α_3 subunits. The data represent the mean±SEM of three to six separate experiments with cells from different donors. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.005$ for differences between groups as indicated

cells showed a homogenous reaction throughout the cytoplasm and cell surface. Immunostaining for integrin α_3 subunit showed a similar reaction in non-endocrine cells (not shown).

Our previous studies have shown that Lu, either alone or together with integrin, mediates the adhesion of mesangial cells [25] and corneal epithelial cells [26] to Lm-511. We therefore studied in more detail the adhesion of dissociated islet cells on Lm-511. Short-term adhesion experiments revealed that cell substratum-coating with purified Lm-511 significantly enhanced adhesion of total islet cells, insulin-positive beta cells and chromogranin-A-positive endocrine cells. Adhesion was inhibited by preincubation with soluble Lu protein, this being as effective as incubation with monoclonal antibodies against α_3 and β_1 integrin subunits (Fig. 6). Notably, soluble Lu inhibited the binding of insulin-positive cells much more effectively than total islet cells (79±4 vs 36±5% inhibition, $n=6$, $p<0.001$).

α5 Lms and Lu in mouse islets of Langerhans Our results on human tissues differ distinctly from earlier mouse studies, which have shown a single-layered Lm α5-containing BM in the vessels of islets [11, 13] and lack of a Lm-511-containing BM in exocrine acini [12]. We therefore reinvestigated the Lm content of mouse islets of Langerhans using two independent new polyclonal antibodies against defined recombinant domains of mouse Lm α5 chain, domains αV and αVI [31]. The results show that vessels were widely labelled in the islets (Fig. 7a,b), whereas at the periphery of the islets occasional streaks of immunoreactivity were found. In line with these findings, no reactivity for Lu was found on endocrine islet cells, although weak vascular labelling was found in- and outside the islets (Fig. 7c).

Discussion

Several studies have suggested that ECM and/or BM proteins are required for differentiation of beta cells [32–34], survival of islets in culture [15, 18] and enhancement of insulin production [35, 36]. However, studies of mice have shown that there is no BM in islets of Langerhans, with the exception of vascular BM [11–14]. Therefore, in the latter studies, it was hypothesised that the vascular BM containing Lm-411 would provide a niche for insulin gene expression for beta cells. Here, we present multiple lines of evidence for a distinctly different duplex BM organisation of human islets of Langerhans.

This evidence is based on: (1) the use of a general (EHS) Lm immunoreactivity; (2) specific profiling of the Lm chain composition of the two distinctively different BMs; (3) demonstration of the duplex BM organisation using

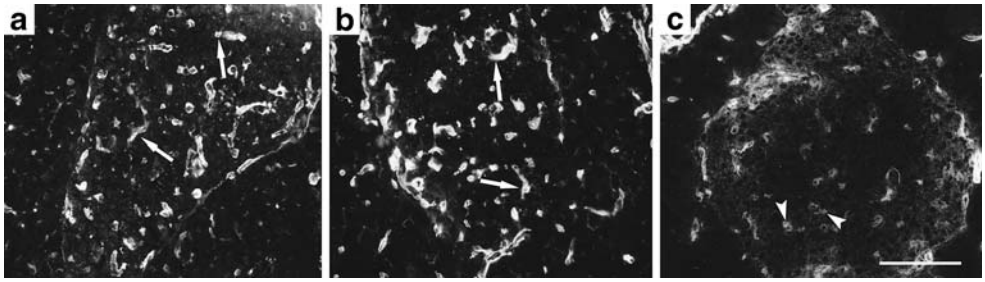

Fig. 7 The distribution of Lm α5 chain (**a**, **b**) and Lu (**c**) immunoreactivities in mouse islets of Langerhans. Polyclonal antisera against Lm α5 domains VI (**a**) and V (**b**) showed that vessels in islets were widely labelled, whereas at the islet periphery occasional streaks of immunoreactivity were seen (arrows). Polyclonal antibodies against Lu showed **c** a faint diffuse granular staining on endocrine cells and more distinct labelling of vessel endothelia (arrowheads). All three reactions indicate a lack of BM organisation in endocrine tissue of mouse islets of Langerhans. Bar, 10 μm

nidogen and collagen type IV as two additional and independent BM markers; (4) ultrastructural demonstration of duplex BM; and (5) demonstration of two specific and distinct sets of cellular receptors to Lm in islets, both in vivo and in vitro. The Lm-511 containing peri-islet BM of the endocrine parenchymal tissue co-invaginates into the islet tissue with vessels and forms a protective sheath around the external walls of the vascular channels. In addition, vascular endothelial cells of the afferent arterioli and capillaries inside the islet are supported by a regular vascular BM. However, it should be noted that on the basis of these morphological results, we cannot conclude which cells produce proteins for parenchymal BM. Our results also suggest that in addition to the classical integrin-Lm receptors, Lu glycoprotein is a prominent Lm receptor on human islet cells, showing a polarised distribution towards the endocrine BM and also being prominent on islet endocrine cells in short-term culture.

In earlier studies, only mouse pancreas has been studied in detail with respect to its Lm content. Jiang et al. [11] showed chains of Lm-211 and Lm-411 in BMs of the acinar tissues. They also showed chains of Lm-411 and Lm-511 around BMs of blood vessels, but could not show any Lms in BMs of pancreatic islets as such. We have now confirmed with two independent antisera that, in line with the results of Miner et al. [12], BMs of mouse exocrine pancreas do not contain Lm-511, while the blood vessels of mouse islets do. This is in contrast to the suggestions of Jiang et al. [11], but in line with those of Nikolova et al. [13].

We also studied the presence of the putative integrin and non-integrin receptors for Lm-511/-521, which comprise integrins $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$ and $\alpha_6\beta_4$, $\alpha_v\beta_3$, dystroglycan protein complex and the Lu blood group antigen, as reviewed [10, 30]. Our results show that in adult human islet tissue immunoreactivities for both integrin β_1 and α_3 are diffusely distributed on endocrine cells without any apparent polarisation towards BM [37] and that immunoreactivities for dystroglycan showed a typical punctate pattern. Remarkably, Lu was very prominent in polarised distribution in the most peripherally located islet cells, strongly suggesting that it is the major Lm-511/-521 binding receptor of endocrine cells. While only little is known about the specific functions of Lu [30], it has been shown that mesangial cells organise the glomerular capillaries via α5-Lm–Lu–integrin interactions [25], suggesting that such interactions may also function at the endocrine–exocrine interface in the human pancreas. Direct evidence for the role of Lu in the anchorage of endocrine cells to Lm-511 has come from cell adhesion studies. Our earlier studies on Lu have shown that while Lu alone cannot mediate the adhesion of mouse mesangial cells [25], it does mediate the adhesion of human endothelial cells [38] together with integrin to Lm-511 and independently of integrins during adhesion of human corneal epithelial cells [26]. The present results show that human islet endocrine cells adhere to Lm-511 in a process inhibited efficiently by soluble Lu, but also by β_1-integrins, presumably integrin $\alpha_3\beta_1$. We cannot exclude the possibility that the adhesion properties of the islet cells were modified by the 2 to 3 day culture period. In particular, this could have affected the polarised localisation of Lm receptors, if the cells had been separated from their natural BM. Further studies are needed to investigate preservation of the human islet BM during

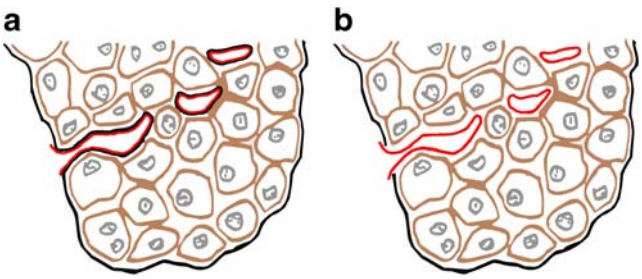

Fig. 8 A schematic view of BM organisation in human (**a**) and mouse (**b**) islets. Black line, endocrine BM, which is seen to co-invaginate with vessel BM (red) into the interior of islet. In mouse only red vessel BM penetrates the islet tissue

islet isolation using collagenase treatment. This has potentially important implications for clinical islet transplantation.

Lm-521 is a Lm isoform found only in some specific tissues such as in kidney glomeruli, synaptic BM and walls of vessels [39]. In this respect it is interesting that we found a high content of Lm β2 chain, a component of Lm-421 and Lm-521, in adult islet vessel BMs and also in BM adjacent to endocrine cells inside the islet.

In summary, we have shown that in clear contrast to the mouse islet, where endocrine cells are in direct contact with the vascular BM, the human islet has a unique double BM structure consisting of specifically structured and closely associated parenchymal and endothelial BMs (for schematic depiction of the BM organisation of islets of Langerhans in man and mouse, see Fig. 8). Furthermore, one of the major Lm receptors found on human islet cells is the Lu glycoprotein. These observations have potentially important implications for the ECM-mediated regulation of human islet cells, as well as for the inflammatory processes associated with insulitis.

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