Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2012.04645.x

Extracellular factors and immunosuppressive drugs influencing insulin secretion of murine islets

V. J. Auer,[1]* E. Janas,[1]* V. Ninichuk,* E. Eppler,† T. S. Weiss,‡ S. Kirchner,§ A. M. Otto* and M. J. Stangl¶

*Institute of Medical Engineering, Technische Universität München (IMETUM), Garching, ‡Center for Liver Cell Research, Department of Pediatrics and Adolescent Medicine, University of Regensburg Hospital, §Hepacult GmbH, Biopark Regensburg, Regensburg, and ¶Chirurgische Klinik und Poliklinik, Transplantationszentrum, Klinikum Großhadern, Ludwig-Maximilians-Universität, München, Germany, and †Research Group Neuro-endocrine-immune Interactions, Anatomisches Institut, Zentrum für Integrative Humanphysiologie (ZIHP), Universität Zürich, Zürich, Switzerland

Accepted for publication 28 June 2012
Correspondence: V. J. Auer, Department Biologie II, Division of Neurobiology, Ludwig-Maximilians-Universität München, Grosshaderner Strasse 2, 82152 Planegg-Martinsried, Germany.
E-mail: veronika.auer@mytum.de

[1]These authors contributed equally to this work.

Summary

Approximately 60% of transplanted islets undergo apoptosis within the first week post-transplantation into the liver attributed to poor engraftment, immune rejection and toxicity of immunosuppressive drugs. Understanding how extracellular matrix (ECM) components, immunosuppressive drugs and proinflammatory cytokines affect insulin secretion will contribute to an improved clinical outcome of islet transplantations. In this study, functional activity of isolated murine islets was measured by glucose-stimulated insulin secretion (GSIS) and by electrophysiological measurements using patch-clamp. Cultivating islets with soluble fibronectin or laminin, as opposed to with coated laminin, markedly increased GSIS. Addition of cyclosporin A reduced GSIS and suppressed glucose-induced spike activity. Tacrolimus affected neither GSIS nor spike activity, indicating a different mechanism. To evaluate the influence of proinflammatory cytokines, islets were incubated with interleukin (IL)-1β, tumour necrosis factor (TNF)-α or with supernatants from cultured Kupffer cells, the main mediators of inflammation in the hepatic sinusoids. IL-1β exerted a bimodal effect on insulin secretion, stimulating below 2 ng/ml and suppressing above 10 ng/ml. Soluble laminin in combination with a stimulatory IL-1β concentration further increased insulin secretion by 20% compared to IL-1β alone, while with high IL-1β concentrations soluble laminin slightly attenuated GSIS inhibition. TNF-α alone did not affect GSIS, but with stimulatory IL-1β concentrations completely abolished it. Similarly, supernatants derived from Kupffer cells exerted a bimodal effect on GSIS. Our data suggest that improved insulin secretion of transplanted islets could be achieved by including soluble laminin and low IL-1β concentrations in the islet cultivation medium, and by a simultaneous inhibition of cytokine secretion from Kupffer cells.

Keywords: cytokines, extracellular matrix, IL-1β, immunosuppressive drugs, insulin secretion, islet transplantation, laminin

Introduction

Beta cell replacement via transplantation of pancreatic islets into the liver is a promising approach for restoring insulin secretion in patients with severe diabetes mellitus [1]. However, approximately 60% of transplanted islets undergo apoptosis within the first week after transplantation, and only a few transplant recipients achieve a normal blood glucose level for more than a few months [2,3]. Encouraging progress was achieved by the Edmonton group using a less diabetogenic and glucocorticoid-free immunosuppressive regimen and a higher number of donor islets [4]. Nevertheless, a 5-year follow-up manifests the need for further improvements in islet survival [5]. The main limitations for long-term insulin independence are inefficient initial neovascularisation and presumed β cell toxicity of immunosuppressive drugs, as well as inflammatory allo- and/or autoimmune responses [6].

The procedure of islet isolation disrupts the vascular connections and thereby the delivery of oxygen and nutrients. Major efforts are directed towards revascularization and maintenance of islet–matrix interactions to improve islet

survival [7,8]. One approach is encapsulation of islets, thereby creating a protective barrier against cellular host response [9]. Such polymer scaffolds have been modified with components of the extracellular matrix (ECM), which are known to support islet function through interactions with ECM receptors [10]. Receptor composition of β cells indicates that most interactions are mediated via laminin [11]. Indeed, laminin and fibronectin appear to improve islet functionality [7,11,12].

Despite the overall beneficial effects of immunosuppression on islet grafts, immunosuppressive drugs are likely to play a significant role in islet destruction due to their presumed β cell toxicity. Intrahepatically transplanted islets are particularly exposed to high drug concentrations in the portal vein [13,14]. The calcineurin inhibitors cyclosporin A (CsA) and tacrolimus are potent immunosuppressive drugs widely used following organ transplantation. However, the use of these drugs is suspected to predispose for post-transplantation diabetes mellitus (PTDM) [15].

The immunological response of the liver is effected by Kupffer cells [16], which are stimulated by transplanted islets to secrete proinflammatory cytokines such as interleukin-(IL)-1β and tumour necrosis factor (TNF)-α. IL-1β appears to be the most important cytokine for islet destruction [17–19]. It is produced not only by activated Kupffer cells [20] and neutrophils [21] but also, to a lower extent, by islet-resident lymphoid, ductal and endothelial cells, and by β cells themselves [22].

The aim of this study was to investigate extracellular factors which may affect the outcome of islet transplantation. Using intact murine islets, we investigated conditions in which ECM components such as laminin and fibronectin could support insulin secretion. Because islets are exposed to immunological stress induced by the transplantation regimen, immunosuppressive drugs and proinflammatory cytokines produced by Kupffer cells were tested for their effects on islets vitality, changes in early membrane-associated processes and insulin secretion.

Materials and methods

Chemicals

Laminin was purchased from Roche (Mannheim, Germany), fibronectin from BD Bioscience (Heidelberg, Germany), CSA and tacrolimus from Sigma-Aldrich (Schnelldorf, Germany), recombinant IL-1β and TNF-α from Invitrogen (Karlsruhe, Germany); other chemicals were purchased from Biochrom AG (Berlin, Germany) and Merck (Darmstadt, Germany).

Islet isolation and cultivation

Pancreatic islets were isolated from inbred wild-type mice on a C57BL/6 background by a standard collagenase digestion [23]. Isolated islets were hand-picked and maintained in culture medium (RPMI-1640 with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin) overnight in a CO_2-incubator.

Kupffer cell isolation and preparation of supernatants

Kupffer cells (Hepacult GmbH, Regensburg, Germany) were obtained from human liver tissue by a modified two-step ethyleneglycol tetraacetic acid (EGTA)/collagenase procedure followed by a Nykodenz density barrier separation and seeded on uncoated cell culture dishes [24,25]. Tissue samples from liver resection were obtained from patients undergoing partial hepatectomy for metastatic liver tumours of colorectal cancer, and non-affected tissue was used for cell purification. Experimental procedures were performed according to the guidelines of the charitable state-controlled foundation Human Tissue and Cell Research, with the patient's informed consent approved by the local ethical committee of the University of Regensburg.

Extracellular matrix and islet seeding

Islets were cultured in surface-activated 24-well culture plates (TPP, Trasadingen, Switzerland) in RPMI medium with 10 islets per well: (1) on uncoated surface; (2) on laminin-coated surface (3 μg/cm^2); (3) seeded on uncoated surface and with soluble laminin (3 μg/ml) added 2·5 h after plating, and (4) as in (3) but with soluble fibronectin (3 μg/ml). After 48 h islets were analysed for GSIS.

Incubation of islets with cytokines or with supernatant from Kupffer cells

Ten islets were seeded per well of a 24-well plate and cultured with increasing concentrations of recombinant IL-1β and/or TNF-α (0·2 ng/ml to 100 mg/ml) in RPMI medium alone, with soluble laminin (3 μg/ml), or on a laminin-coated (3 μg/cm^2) surface for 24 h. For their stimulation, isolated Kupffer cells (10^6 per well) were cultured in 24-well plates in RPMI-1640 without FCS and with lipopolysaccharide (LPS) (10 μg/ml) added for 24 h. Other treatments are as indicated in the figure legends. The IL-1β concentration was determined by enzyme-linked immunosorbent assay (ELISA) (Invitrogen, Darmstadt, Germany).

Insulin secretion

Islets were first incubated for 30 min in Krebs–Ringer-bicarbonate (KRB) buffer containing 2·8 mM glucose, and then for 1 h in KRB buffer with 2·8 mM glucose (basal insulin secretion) and 1 h with 16·8 mM glucose (GSIS). Supernatants were analysed for insulin content by ELISA (Mercodia, Uppsala, Sweden). To determine the acute effects of immunosuppressive drugs on GSIS, the drugs were added

at the concentrations indicated to the second incubation step (16·8 mM glucose). Stimulation indices were calculated as the ratio of GSIS (16·8 mM glucose) to basal insulin secretion (2·8 mM glucose).

Live/dead staining

Live/dead fluorescent staining was performed by incubating islets with 15 μM propidium iodide and 10 μM acridine orange [26]. Cells were imaged with a fluorescent microscope (Axiovert, Zeiss, Jena, Germany).

Adenosine triphosphate (ATP) content

Determination of the ATP content of islets was adapted from Giroix *et al.* [27]. Isolated islets were incubated in KRB supplemented with increasing concentrations of CsA (10–40 μM) for 1 h at 37°C and washed with KRB buffer. Ten islets were incubated for 5 min at 37°C in 360 μl KRB without glucose or with 16 mM glucose. The incubation was stopped on ice and adenine nucleotides extracted with 120 μl NaOH (160 mM), 6·0 mM ethylenediamine tetraacetic acid (EDTA) and Triton X-100 (0·0004%, w/v). After 5 min on ice and 5 min centrifugation at 1000 ***g***, the supernatant was neutralized with 30 μl HCl (650 mM). To all tubes, 120 μl KRB buffer was added and ATP was determined in 50-μl aliquots using the ATP Bioluminescence Assay KIT CLS II (Roche Diagnostics, Mannheim, Germany).

Electrophysiological measurements

Patch clamp experiments were performed in the perforated patch mode using an EPC 10 patch clamp amplifier (HEKA Electronics, Lambrecht, Germany). Changes in membrane potential were recorded in the current-clamp mode with amphotericin B (250 μg/ml) in the pipette solution containing (mM): 10 NaCl, 10 KCl, 70 K_2SO_4, 4 $MgCl_2$, 2 $CaCl_2$, 10 EGTA and 5 HEPES, and at pH 7·15 adjusted with KOH. The pipette resistance ranged from 3 to 7 MΩ when filled with pipette solution. The standard bath solution contained (mM): 140 NaCl, 3·6 KCl, 2 $NaHCO_3$, 0·5 $MgSO_4$, 0·5 NaH_2PO_4, 1 $CaCl_2$, 5 HEPES and glucose as indicated; pH 7·4 was adjusted with NaOH. Membrane potential was measured at 30°C. For recording of Ca^{2+} currents, the pipette solution consisted of (mM) 76 Cs_2SO_4, 10 NaCl, 10 KCl, 1 $MgCl_2$ and 5 HEPES; pH 7·35 was adjusted with KOH. To block outward currents, 20 mM tetra-ethylammonium chloride was added to the standard bath solution (NaCl was reduced to maintain iso-osmolarity). Glucose was added at 5 mM. Na^+ currents were blocked by addition of 1 μM tetrodotoxin. Current measurements were carried out at room temperature. Only experiments with a series resistance lower than 30 MΩ were analysed. Beta cells were identified according to their steady-state inactivation of Na^+ channels [28]. Particular voltage protocols are described in the Results.

Data analysis

For data analysis and figure preparation, OriginPro8 (Original Lab Corp., Northampton, MA, USA) and Excel (Microsoft, Redmond, WA, USA) were used. Data are means ± standard error of the mean (s.e.m.). Statistical significances were evaluated by Student's paired or unpaired *t*-tests, as appropriate. Differences were considered significant at $P < 0{\cdot}05$.

Results

Extracellular matrix and insulin secretion

To investigate how ECM could sustain β cell function, islets were cultured on a laminin-coated surface or with soluble laminin or soluble fibronectin. Basal as well as glucose-stimulated insulin secretion (GSIS) were lowest for islets maintained on a laminin-coated surface compared to those on an uncoated surface, whereas islets cultured with soluble laminin showed a threefold increase in basal and in GSIS (Fig. 1a). Soluble fibronectin induced a 2·5-fold increase in GSIS. Our data indicate that soluble matrix components support the secretory function of β cells. Cell viability and islet morphology were sustained in all conditions, excluding cell death as parameters for differences in insulin secretion (Fig. 1b–e).

Effects of immunosuppressive drugs

To test CsA and tacrolimus for acute effects on GSIS, islets were exposed for 1 h to these drugs during incubation with high glucose. In islets treated with 5–10 μM CsA, insulin secretion was attenuated significantly, on the average by about 55% compared to dimethylsulphoxide (DMSO)-treated islets, while only a small effect was observed with 1 μM CsA. Tacrolimus at 1–4 μM did not, or only marginally, impair GSIS compared to the control (average stimulation indices: 4·3 ± 0·9 for DMSO and 4·6 ± 0·8 for 2 μM tacrolimus). Thus, even though both drugs are calcineurin inhibitors, they had divergent effects on GSIS.

To decipher possible mechanisms for these differences, CsA and tacrolimus were investigated for their effects on glucose-induced oscillations of membrane potential, which correlate with insulin secretion and intracellular Ca^{2+} concentration [30,31]. Stimulation of β cells with 10 mM glucose resulted in characteristic spike activity (Fig. 2b,c), which was not affected by the addition of CsA up to 4 μM (data not shown). However, increasing CsA concentrations up to 10 μM abolished electrical activity (Fig. 2c), which is in line with reduced insulin secretion (Fig. 2a). After the drug washout, spike activity was restored. In contrast, addition of 4 μM tacrolimus did not alter glucose-stimulated electrical activity (Fig. 2b), which agrees with the lack of effect on GSIS (Fig. 2a). These results point further to different modes of action of these two drugs on GSIS.

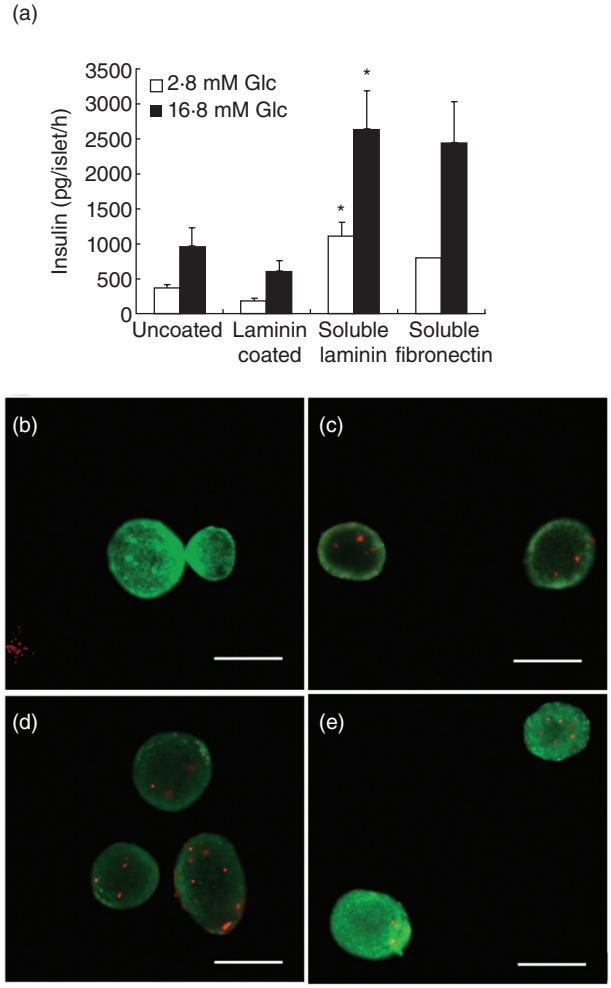

Fig. 1. Effects of extracellular matrix (ECM) on glucose-stimulated insulin secretion. (a) Islets were cultured for 48 h on an uncoated surface, on a laminin-coated surface (3 μg/cm^2) or in the presence of soluble laminin (3 μg/ml) or soluble fibronectin (3 μg/ml). Insulin secretion was determined in response to 2·8 mM glucose (white bars) and 16·8 mM glucose (black bars). Data are expressed as the means ± standard error of the mean of four to five samples. Differences between control and treated groups were tested for significance. Differences between control and soluble laminin-treated groups were statistically significant, with $P = 0{\cdot}01$ for basal secretion and $P = 0{\cdot}03$ for stimulated insulin secretion. (b–e) After insulin determination, islet viability was assessed by fluorescent double staining with propidium iodide (red) as an exclusion dye and acridine orange (green) as an inclusion dye for living cells. Islets incubated (b) on an uncoated surface, (c) on a laminin-coated surface, (d) with soluble laminin and (e) with soluble fibronectin. Bar: 100 μm.

Because spike activity and insulin release are triggered by Ca^{2+} influx, CsA was tested for its effects on voltage-dependent Ca^{2+} channels. It has been suggested that CsA impairs insulin release by interfering with the voltage-dependent Ca^{2+} transport [32]. However, we observed no differences in the recordings of Ca^{2+} currents elicited before and after addition of 10 μM CsA (Fig. 3a). The current/voltage relationship (Fig. 3b) for CsA-treated and untreated β cells shows that the peak current amplitude and voltage dependence overlap for all conditions. Apparently, CsA does not immediately affect Ca^{2+} influx through Ca^{2+} channels.

ATP-sensitive K^+ (K_{ATP}) channels serve as metabolic sensors that convert changes in cell metabolism into changes of electrical activity and, therefore, have a major role in stimulus-secretion coupling [33]. To elucidate whether CsA inhibits spike activity by interfering with K_{ATP} channels, sulphonylurea tolbutamide, a pharmacological blocker of K_{ATP} channels, was applied. After glucose stimulation and subsequent inhibition of spike activity by 10 μM CsA, tolbutamide at 100 μM restored spike activity in β cells (Fig. 3c). As tolbutamide overcomes inhibition by CsA, this points to an interaction upstream of or directly at the K_{ATP} channel. A hallmark of K_{ATP} channels is their sensitivity to inhibition by increasing the intracellular ATP concentration [33]. However, after treatment with different CsA concentrations, the ATP content of β cells was not changed (Fig. 3d). Thus, CsA did not affect K_{ATP} channels by depleting ATP.

Bimodal effect of IL-1β on insulin secretion

To test the effect of proinflammatory cytokines, islets were cultured with increasing concentrations of IL-1β and/or TNF-α (Fig. 4). IL-1β had a bimodal effect: at 0·2 ng/ml it induced a 50% increase in GSIS compared to control, while IL-1β above 10 ng/ml led to a progressive decline in GSIS (Fig. 4a). To investigate whether this stimulating effect could be enhanced further by laminin, islets were cultured with IL-1β alone or in combination with soluble laminin or on a laminin-coated surface for 24 h (Fig. 4b). With soluble laminin alone we observed a 30% increase in GSIS compared to control. This effect was smaller than that in Fig. 1, which might be explained by the shorter incubation time of 24 h. The combination of soluble laminin with IL-1β at 0·02 ng/ml produced an additive stimulatory effect on GSIS. Little further enhancement was observed with IL-1β at 0·2 ng/ml. With an inhibitory IL-1β concentration (20 ng/ml), soluble laminin slightly attenuated the inhibitory effect of IL-1β. In contrast, when islets were cultured on a laminin-coated surface, GSIS was reduced by about 45% compared with the uncoated control. However, stimulatory IL-1β concentrations still were able to improve GSIS. Taken together, soluble laminin in combination with IL-1β had an additive effect on GSIS.

Treatment with TNF-α alone did not affect GSIS (Fig. 4c), but completely abolished GSIS with stimulatory IL-1β concentrations (Fig. 4d). Under all conditions, viability was not impaired (Fig. 4e–i).

To assess whether Kupffer cells, the main source of proinflammatory cytokines in the liver, affect insulin secretion, we tested supernatants from human Kupffer cells on murine islets. Earlier studies on rat islets with human IL-1β have

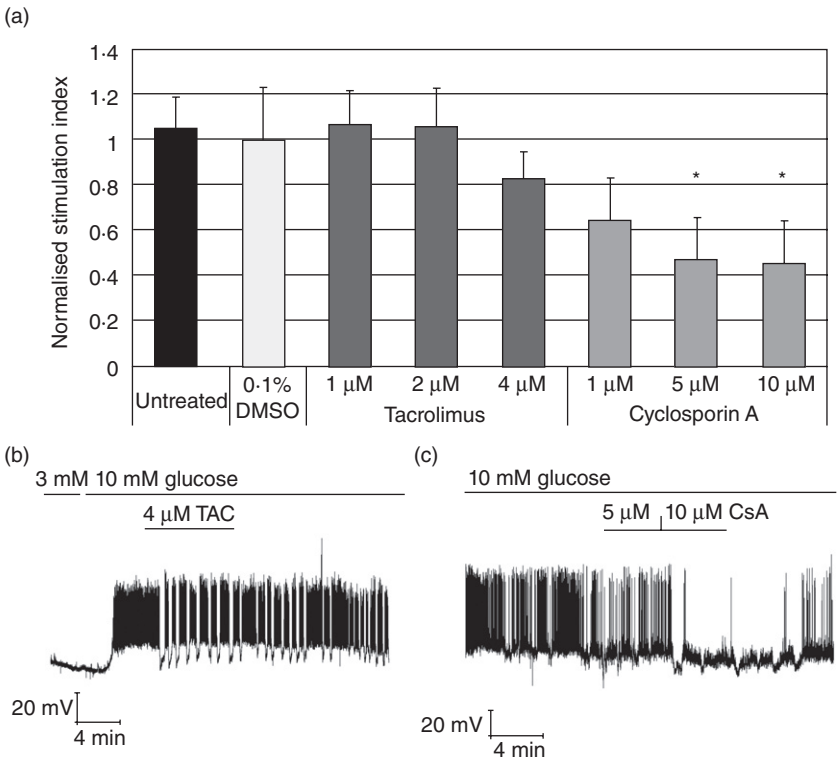

Fig. 2. Effects of the immunosuppressive drugs cyclosporin A (CsA) and tacrolimus (TAC) on islet activity. (a) Glucose stimulation index was normalized to 1 for the dimethylsulphoxide (DMSO)-treated control. Values are the means ± standard error of the mean of nine to 12 samples from three separate experiments ($P < 0{\cdot}05$). (b,c) Representative recordings of time-resolved oscillations of membrane potential in the presence of low glucose (3 mM), after challenge with glucose (10 mM) and after application of (b) 4 μM tacrolimus or (c) 5 μM CsA and 10 μM CsA, followed by washout. Of seven cells measured, the electrical activity was already abolished in two cells treated with 5 μM CsA, while it was sustained in one cell treated with 10 μM CsA.

shown that IL-1β action is preserved between humans and rodents [34,35]. Islets treated with supernatants from non-stimulated Kupffer cells, containing 9·1 pg/ml IL-1β, showed an increase in GSIS (Fig. 5a). To stimulate cytokine production, Kupffer cells were incubated with 10 μg/ml LPS for 24 h [36]. To exclude a direct effect of LPS on insulin secretion (since β cells possess LPS-binding Toll-like receptors [37]), LPS was added directly to the islets; no effect on GSIS was observed (Fig. 5b). However, islets treated with supernatants from LPS-activated Kupffer cells, containing 919 pg/ml IL-1β, showed a markedly reduced GSIS. Thus, a bimodal effect was observed also with Kupffer cell supernatants.

Supernatants from non-stimulated Kupffer cells cultured with CsA contained a stimulatory IL-1β concentration

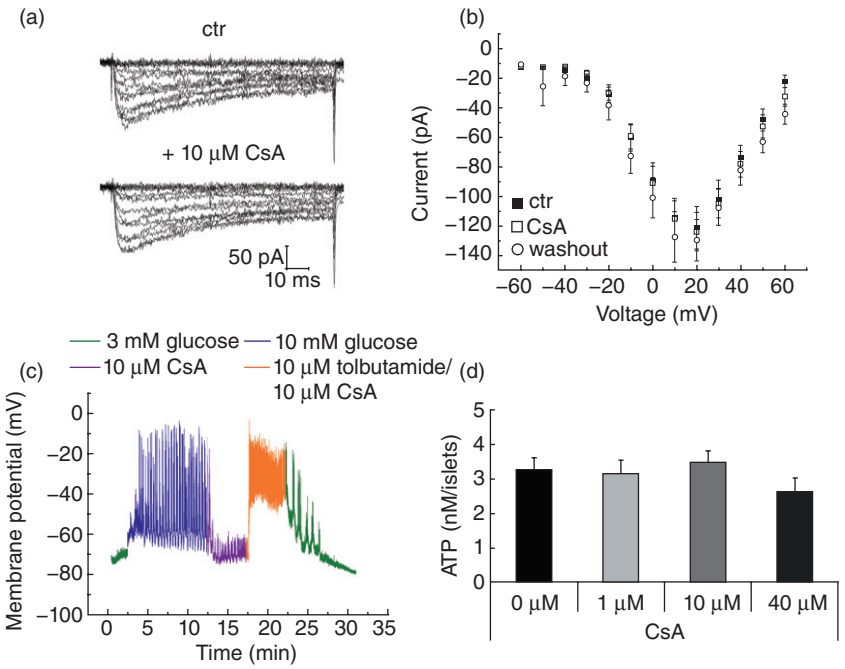

Fig. 3. Effects of cyclosporin A (CsA) on voltage-gated calcium channels and adenosine triphosphate (ATP) levels. (a) Representative recordings of whole-cell calcium currents elicited by a voltage-clamp depolarization from −60 mV to +60 mV in 10-mV increments under control conditions (upper panel) and after addition of 10 μM CsA (lower panel). (b) Plot of the current–voltage relationship under control conditions (■), after application of CsA (□) and after washout (○). Data represent the means ± standard error of the mean (s.e.m.) of eight experiments. (c) Time-resolved recording of oscillations of membrane potential in the presence of 3 mM glucose, after challenge with 10 mM glucose, addition of CsA (10 μM) and after addition of the sulphonylurea tolbutamide (100 μM). The trace is representative of seven similar recordings from five independent experiments. (d) Cellular ATP content after 1 h incubation with different CsA concentrations. Data represent the means ± s.e.m. for four to 11 samples.

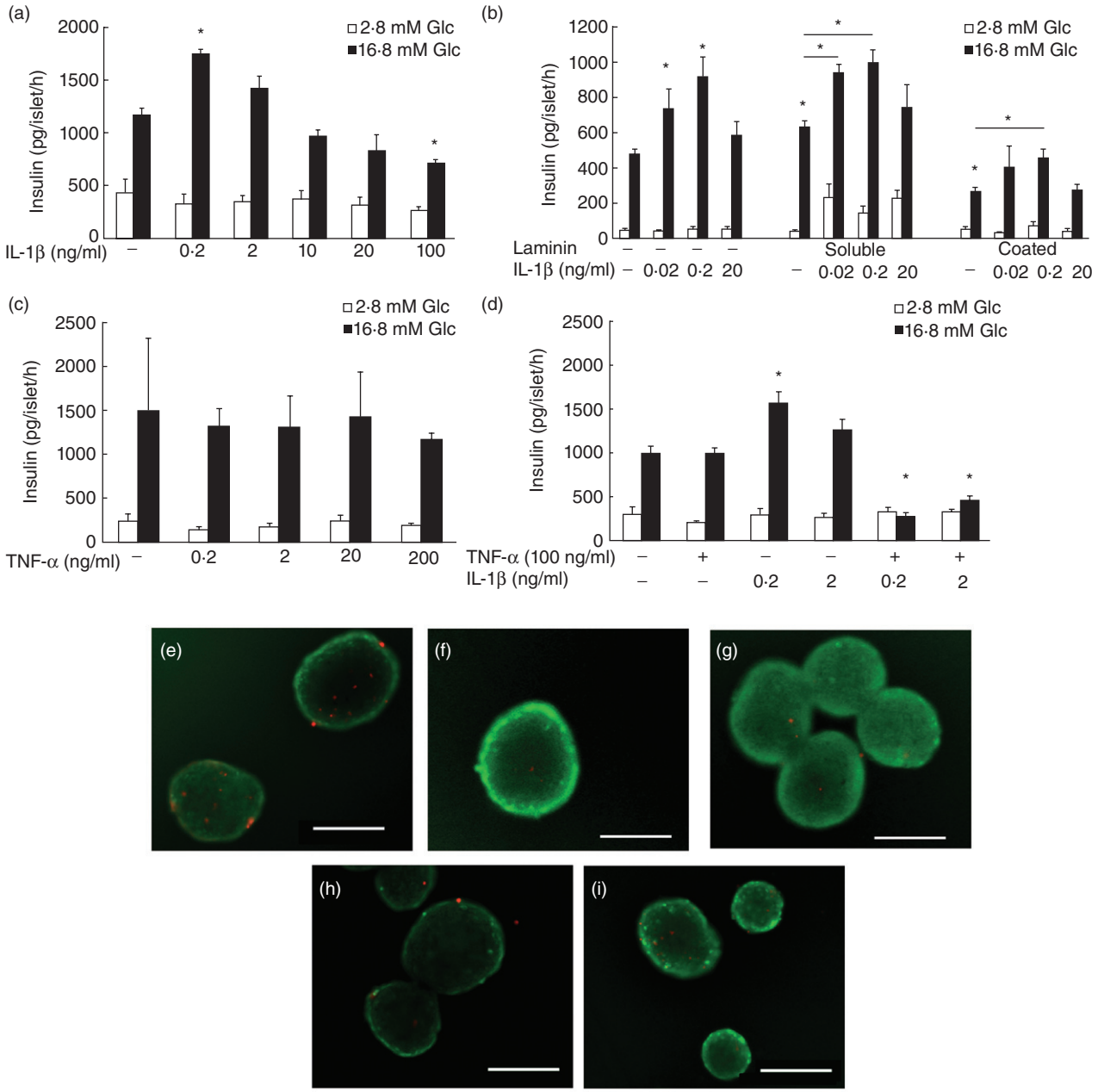

Fig. 4. Effects of cytokines on glucose-stimulated insulin secretion. Islets were cultured for 24 h with increasing concentrations of (a) interleukin (IL)-1β alone or (b) IL-1β in different culture conditions (soluble laminin *versus* laminin-coated), (c) tumour necrosis factor (TNF)-α alone or (d) TNF-α with IL-1β. Insulin secretion was determined after a 1-h incubation at 2·8 mM glucose (white bars) followed by an additional incubation at 16·8 mM glucose (black bars). (a) The diagram is representative of four independent experiments, each with $n = 3$. Results are significantly different between controls and treatments with 0·2 ng/ml IL-1β ($P = 0{\cdot}01$) and with 100 ng/ml IL-1β ($P = 0{\cdot}007$). (b) Data are expressed as the means ± standard error of the mean (s.e.m.) for three to six samples from two independent experiments ($P < 0{\cdot}05$). (c,d) Data are expressed as the means ± s.e.m. for three to six samples ($P < 0{\cdot}05$). (e–h) After insulin determination, viability of islets was assessed by fluorescence double staining. Islets were (e) untreated, or treated with (f) IL-1β (20 ng/ml) and soluble laminin or (g) IL-1β (20 ng/ml) on laminin-coated dishes or with (h) TNF-α (100 ng/ml) or (i) TNF-α (100 ng/ml) and IL-1β (2 ng/ml). Bar: 100 μm.

(13·4 pg/ml). Nevertheless, treating islets with this supernatant resulted in reduced GSIS compared to control. In contrast, supernatants from LPS-stimulated Kupffer cells cultured with CsA contained a high IL-1β level (904 pg/ml) and completely suppressed GSIS (Fig. 5a). These cultivation conditions did not affect viability of β cells (Fig. 5c–e). In summary, CsA was not immediately cytotoxic and did not inhibit secretion of IL-1β by Kupffer cells, but it exerted a strong suppressive effect on GSIS, regardless of the stimulatory effect of IL-1β.

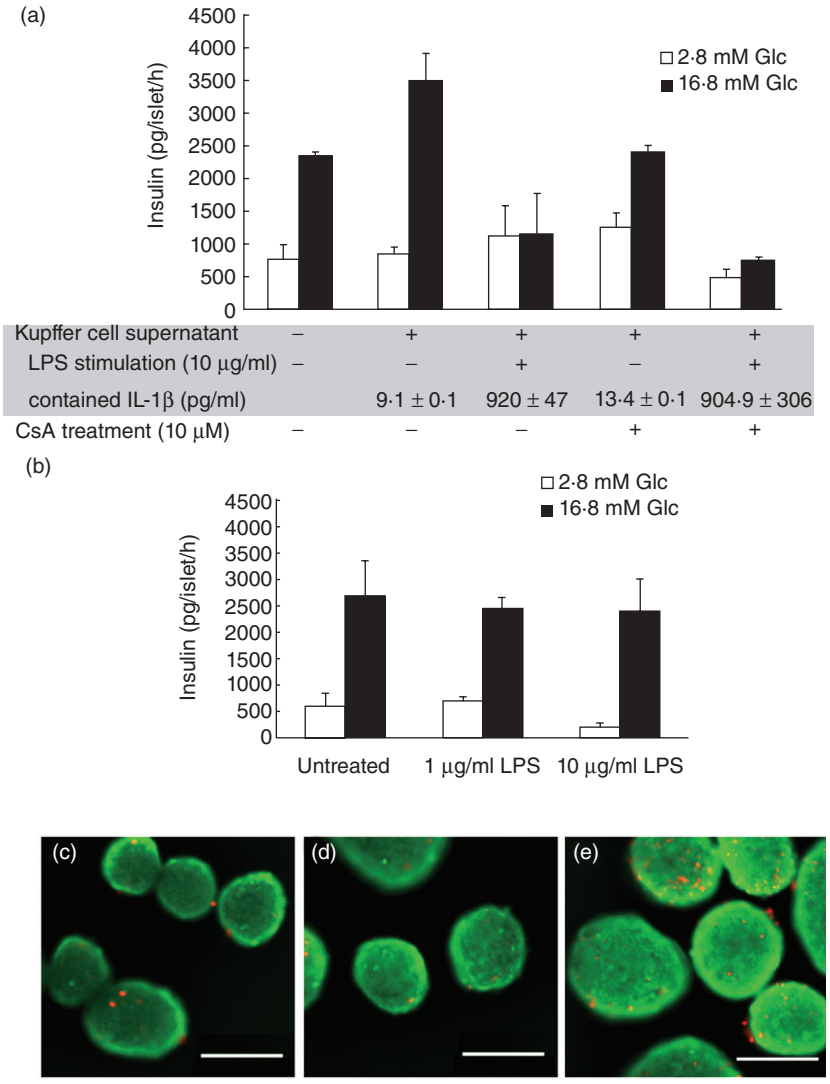

Fig. 5. Effects of supernatants from human Kupffer cells on glucose-stimulated insulin secretion of islets. (a) Islets were treated with supernatants of Kupffer cells preincubated for 24 h without or with lipopolysaccharide (LPS) (10 μg/ml), with cyclosporin A (CsA) (10 μM) alone or with LPS (10 μg/ml). The respective IL-1β concentrations in the Kupffer cell supernatants are indicated as determined by anti-IL-1β enzyme-linked immunosorbent assay (ELISA). Insulin secretion was determined after a 1-h incubation at 2·8 mM glucose (white bars), followed by subsequent incubation at 16·8 mM glucose (black bars). Data are expressed as the means ± standard error of the mean of three samples. (b) Insulin secretion by islets after treatment with LPS for 24 h ($n = 3$). (c–d) Viability of islets was assessed by fluorescent double staining: islets were (c) untreated, (d) treated with supernatant of non-stimulated, CsA-treated Kupffer cells and (e) treated with supernatant of LPS-stimulated and CsA-treated Kupffer cells. Bar: 100 μm.

Discussion

Limited survival of islet allograft and compromised insulin secretion may be explained by a disturbed islet–matrix interaction and immunological stress induced by the transplantation procedure. Therefore, we have examined how different components, such as ECM, immunosuppressive drugs and proinflammatory cytokines may influence islet functionality.

The composition of the extracellular matrix surrounding islets has been reported to critically influence β cell function [7,8,38]. In particular, laminin, the major component (80%) of the basement membrane, as well as fibronectin have been shown to affect various signalling pathways and regulatory processes required for insulin secretion [29,38–44]. In our experiments, soluble laminin markedly and soluble fibronectin moderately enhanced GSIS, while islets cultivated on a laminin-coated surface released less insulin. An explanation for the different effects of soluble *versus* coated laminin could be a dynamic integrin cell surface expression, as the expression of integrins is known to be up-regulated in the presence of certain ECM proteins [7,45–47]. One could hypothesize that soluble laminin covers the spheroid structure of islets evenly, cross-linking a higher number of integrins compared to coated laminin ('local application'), which accesses only a limited number of receptors on islet cells. Such an islet–matrix interaction could stabilize further intercellular connections (e.g. gap junctions, E-cadherins, etc.), which are important in the transduction of signals related to processes such as glucose sensing and insulin secretion [11].

CsA and tacrolimus have been associated with decreased glucose tolerance, hyperglycaemia and PTDM [15]. Several reports point to adverse effects of CsA and tacrolimus on β cell function *in vitro* and *in vivo* [48–50], but the modes of action remain unresolved. In our study, acute administration of CsA inhibited spike activity and subsequently GSIS. These results are in line with earlier studies showing that, even at

low doses, CsA directly modulates various cellular pathways, e.g. mitochondrial function, and thereby compromises insulin secretion [51–53]. In contrast, tacrolimus affected neither spike activity nor insulin secretion. Other reports have similarly shown no acute effects of tacrolimus on GSIS in cell-lines and islets [54–56]. Nevertheless, long-term *in-vitro* treatment (>48 h) with tacrolimus reduces insulin synthesis and secretion, and induces apoptosis of β cells [54,55,57,58]. Similar results have been observed with CsA after long-term treatment [53,59]. Because both CsA and tacrolimus are calcineurin inhibitors, these effects are probably due to inhibition of the calcineurin/nuclear factor of activated T cells (NFAT) signalling, which regulates β cell mass and function [60]. However, CsA differs from tacrolimus by immediately compromising insulin secretion.

The proinflammatory cytokines IL-1β and TNF-α are highly elevated in serum after transplantation [61]. In addition to the pro-apoptotic role of proinflammatory cytokines, IL-1β has been reported to enhance insulin secretion [62,63]. In line with these findings, in our study we showed a bimodal effect of IL-1β on GSIS. Low IL-1β concentrations are known to be secreted by islets themselves, underlining the physiological role of IL-1β in the regulation of insulin secretion [64].

TNF-α at concentrations up to 100 ng/ml did not affect insulin secretion [65]. However, with high concentrations of IL-1β, TNF-α completely abolished GSIS; this is in line with the reported effect of TNF-α potentiating the IL-1β-induced inhibition of insulin secretion [65]. An unexpected novel result is that even with low IL-1β concentrations TNF-α abolished GSIS, i.e. reversing the stimulatory effect of IL-1β to inhibition. A possible explanation may be based on the fact that both IL-1β and TNF-α are known to activate NF-κB signalling [66,67]. We hypothesize that the concentration of NF-κB triggered by IL-1β and TNF-α together exceeds a certain value which in turn influences negatively gene expression required for insulin secretion and, rather, up-regulates apoptotic genes.

Soluble laminin together with stimulatory IL-1β concentrations exerted an additive effect on insulin secretion, whereas soluble laminin with an inhibitory IL-1β concentration slightly reduced IL-1β-induced inhibition. This confirms a previous report demonstrating that islets simultaneously cultivated with stimulatory IL-1β and laminin (804G_ECM) showed an increased expression of the insulin gene compared to cultivation with laminin alone [64].

Besides direct cross-talk of signal molecules activated by ECM components and cytokines, it is worth mentioning that islet behaviour is also modulated indirectly by the extracellular matrix. The ECM selectively binds cytokines and other growth factors, thereby protecting them from degradation, and increases their local concentration and availability for their receptors [68].

An important finding in this study is the observation that supernatants from Kupffer cells exerted a bimodal effect on insulin secretion. Non-activated Kupffer cells, producing low IL-1β concentrations, supported insulin secretion, while LPS-stimulated Kupffer cells, producing high IL-1β levels and representing a situation likely to occur during transplantation, suppressed GSIS.

To mimic post-transplantation conditions more effectively, CsA was included during LPS stimulation of Kupffer cells. Together with IL-1β produced by Kupffer cells, CsA reduced GSIS markedly, reflecting the dramatic events during transplantation. In animal studies, administration of drugs that inhibited cytokine action or macrophage function improved islet survival [69,70], emphasizing the role of activated macrophages in islet destruction. However, our finding of a stimulatory effect of IL-1β indicates that a complete blockage of IL-1β during post-transplantation therapy (e.g. by application of anti-IL-1β antibodies) might be counterproductive.

Strategies targeting Kupffer cell depletion, thereby decreasing IL-1β and TNF-α concentrations [61,71] while preserving low endogenous IL-1β production by the islets themselves, would improve islet function markedly. Islet engraftment could be promoted further by encapsulation of islets into scaffolds which, when modified by soluble laminins, would increase insulin secretion and in addition serve as a platform for cytokines and angiogenic factors [72,73].

Acknowledgements

This work was supported by the BFS-Bayerische Forschungsstiftung (Project 721/06). Some consumables were financed by a research grant (to E.E.) by Boehringer Ingelheim, Germany.

Disclosure

The authors declare no conflicts of interest.

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