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Expression and function of leukaemia inhibitory factor and its receptor in normal and regenerating rat pancreas

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Abstract *Aims/hypothesis:* It was recently reported that culturing adult exocrine cells in the presence of epidermal growth factor and leukaemia inhibitory factor (LIF) resulted in their transdifferentiation into endocrine beta cells. The aim of this study was to examine the expression and function of LIF in the pancreas. *Materials and methods:* We studied the expression of LIF and its receptor components, LIF-receptor- β and gp130, by immunohistochemistry, western blotting and RT-PCR in normal rat pancreas, pancreas with duct ligation-induced islet neogenesis, and in pancreatic cell cultures. Isolated duct fragments were cultured in the presence of LIF and a janus kinase 2 (JAK2) inhibitor. *Results:* LIF was detected by immunohistochemistry, western blot and RT-PCR in the ducts of the normal pancreas. Both LIF-receptor- β and gp130 were detected by RT-PCR in the pancreas. Immunostaining revealed gp130 exclusively in the ducts and centro-acinar cells. After duct ligation-induced tissue injury, upregulation of LIF and its receptor occurred in rat pancreas. Metaplastic exocrine cells also started to express LIF and this was increased after alloxan treatment. Signalling via LIF-receptor- β /gp130 involves the JAK/signal transducer and activator of transcription (STAT) pathway. LIF induced increased activation of STAT3 in pancreatic cells. In isolated duct fragments, addition of LIF resulted in a significant increase in duct cell proliferation, while a specific inhibitor of the JAK/STAT signalling pathway inhibited proliferation. *Conclusion/interpretation:* Our observations show that LIF and its receptor are expressed in

cells from pancreatic ducts. The cytokine plays a role in pancreatic physiology, controls duct cell proliferation and is involved in repair processes following pancreatic injury.

Keywords Duct cell proliferation · Islet neogenesis · Leukaemia inhibitory factor · Pancreas

Abbreviations BrdU: bromodeoxyuridine · CK20: cytokeratin-20 · EGF: epidermal growth factor · FITC: fluorescein isothiocyanate · IFNG: interferon gamma · JAK: janus kinase · LIF: leukaemia inhibitory factor · LIFR: leukaemia inhibitory factor receptor- β · PAK: polyclonal anti-keratin · STAT: signal transducer and activator of transcription · TRITC: tetramethyl rhodamine isothiocyanate

Introduction

There is currently a lot of interest in the possibility of treating diabetes by cell-based regenerative medicine. Indeed, beta cell mass regulation is a critical issue for understanding diabetes, a disease characterised by a near absolute (type 1) or relative (type 2) deficiency in the number of pancreatic beta cells [1]. In postnatal rodents the slow expansion of the beta-cell mass during physiological conditions can be attributed to the low mitotic activity of pre-existent beta cells [2–4]. The limited and slow regenerative growth of beta cell mass following surgical removal of two-thirds of the pancreas volume is also caused by beta cell mitosis [4]. However, following acute and extensive injury of the exocrine tissue, a rapid and more robust expansion of the endocrine cell mass in the absence of increased beta cell mitotic activity has been attributed to neogenesis from progenitor cells. This is the case, for example, in IFNG transgenic mice [5] and duct-ligated rats [6, 7]. In rats that underwent exocrine tissue injury caused by duct ligation and in which the exocrine cells were further stimulated with the hormone gastrin, a doubling of beta cell mass occurred within only 3 days. In this period, no increased beta-cell mitotic activity could be discerned

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and this activity is far too low to explain the observed numerical increase in beta cells [7]. In mice in which beta cells were depleted by alloxan treatment, administration of gastrin plus epidermal growth factor (EGF) led to regeneration of a functional beta cell mass in only 1 week. Also in this experimental model, mitotic activity of beta cells was far too low to explain the rapid expansion of the beta-cell mass. In this experimental model, a pulse-chase experiment with the thymidine analogue bromodeoxyuridine (BrdU) demonstrated an influx of labelled cells from the exocrine (acinar, duct) to the endocrine cell compartment [8]. Co-expression by intermediate cells of insulin and amylase or cytokeratin-20 suggested transdifferentiation of exocrine acinar and/or ductal cells to beta cells [8, 9]. We recently developed an *in vitro* model to study beta cell neogenesis. In this model we demonstrated that the combination of EGF and leukaemia inhibitory factor (LIF) directly induces the neof ormation of beta cells from exocrine cell cultures that had been previously depleted of contaminating beta cells [10]. Whereas EGF is a ubiquitously expressed growth factor affecting proliferation in most epithelial tissues, including the pancreas [11], virtually nothing is known with regard to LIF in this organ. The importance of cytokines in the regulation of pancreas regeneration has received little attention so far. However, in regeneration models it has been shown that inflammatory mediators are involved. For example, beta-cell regeneration is induced following subtotal pancreatectomy [12, 13], but in the absence of inflammation subtotal pancreatectomy does not lead to beta-cell regeneration [14]. Transgenic mice that overexpress the cytokine IFNG in pancreatic islets show combined beta cell destruction and regeneration, the latter by a process of neogenesis from ductal complexes [5]. In IFNG transgenic mice, transient upregulation of EGF, TGFA and the EGF receptor were observed in acini undergoing transdifferentiation into duct-like structures [15]. Beta cell regeneration from ducts also occurs in transgenic mice with pancreatic overexpression of IL6 [16]. In the present study we investigated the expression and function of LIF in the pancreas. LIF is a 40–45 kDa monomeric glycoprotein belonging to the IL6 family of cytokines. It is a pleiotropic molecule known to induce differentiation of mouse myeloid leukaemic cells [17], regeneration of muscle [18] and nerve [19], and mesenchymal-to-epithelial transdifferentiation in the developing kidney [20], whereas it inhibits differentiation and promotes proliferation of embryonic stem cells [21]. The action of LIF is mediated by a functional LIF receptor composed of two signal-transducing proteins, LIF receptor- β (LIFR) and gp130. Signal transduction after LIF binding to the LIFR/gp130 complex is accomplished preferentially through the janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway. Activation of the LIFR/gp130 complex induces tyrosine phosphorylation of STAT proteins (preferentially STAT3) by receptor-associated JAKs. Phosphorylated STAT proteins subsequently form hetero- or homodimers and trans-

locate to the nucleus, where they exhibit transcriptional activity on target genes [22].

Given the remarkable effect of LIF on *in vitro* beta-cell neogenesis [10], we wanted to get a better understanding of its physiological relevance and to know whether LIF is expressed in the normal pancreas and in injured pancreatic tissue. We also wondered whether the molecular components of the LIF receptor complex and its signalling pathway are expressed in the pancreas.

Material and methods

Animals and animal procedures

Adult male Wistar rats (Janvier, Le Genest-St-Isle, France) of 250–300 g body weight (12 weeks of age) were housed according to the guidelines of the Belgian Regulations for Animal Care. To induce pancreatic regeneration/tissue remodelling, rats were subjected to ligation of the exocrine ducts draining the splenic half of the pancreas, as described previously, and the tissue was collected after 7 days [6]. To collect ductal fluid the main pancreatic duct was occluded for 2 days, after which the fluid was aspirated with a syringe from the swollen duct. The experiments were approved by the Ethical Committee of the Vrije Universiteit Brussel.

Isolation and culture of rat cells

Pancreases from adult rats were dissociated with collagenase and exocrine acinar cells [23] and ducts [24] were isolated and cultured as described. After culture for 4 days in suspension, acinar cells give rise to metaplastic cells with ductal characteristics [23]. For some experiments the cells were incubated for 1 h with 10 mmol/l alloxan to deplete contaminating beta cells [10].

Hand-picked duct fragments were explanted on a collagen layer. The collagen solution consisted of 1.5 g/l collagen R (Serva, Heidelberg, Germany), 1×RPMI (Sigma, St Louis, MO, USA) and 2.3 g/l NaHCO₃, and NaOH was added until a basic colour shift occurred. Aliquots of 250 μ l of collagen solution were transferred into a 24-well plate (Falcon; BD Biosciences, Erembodegem, Belgium), and after polymerisation duct fragments were seeded on the collagen in RPMI-1640 with Glutamax-1 (Invitrogen, Carlsbad, CA, USA) containing 1,000 U/ml penicillin, 1,000 μ g/ml streptomycin (Bio-Whittaker, Verviers, Belgium) and 10% FCS (Perbio, Erembodegem, Belgium). When ducts adhered, medium was replaced by medium containing 1% FCS with or without 40 ng/ml LIF (Sigma) or 100 μ mol/l AG490 (Calbiochem, San Diego, CA, USA) or a combination of both for 24 h. Six hours before harvesting the explants, 10 μ mol/l BrdU (Sigma) was added. Collagen was digested with 0.3 g/l collagenase P (Roche, Mannheim, Germany) followed by 4% formaldehyde fixation and embedding in paraffin for sectioning.

RT-PCR

Pancreatic total RNA was extracted with TRIzol (Invitrogen) after incubation of the tissue in RNA later (Sigma) to improve RNA quality. RNA from cells was extracted after incubation in RNA later, with GenElute mammalian total RNA miniprep kit (Sigma). All samples were checked for RNA degradation on a Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Only samples with good RNA quality were used for RT-PCR. cDNA was prepared by reverse transcription on 500 ng total RNA. PCR ($n=3$) was performed with Platinum *Taq* DNA Polymerase (Invitrogen). The primer pairs for rat *Lif* were: forward 5'-CATGACGG ATTTCCCACCTTT-3' and reverse 5'-GCAGCCCAACT TCTTCCTTTG-3'. Those for rat *Lifr* were: forward 5'-TTGGAAATACGTTCCGCCCT-3' and reverse 5'-GGAA CACTGCCGATTTTC-CTG-3'. Those for rat *gp130* were: forward 5'-TCCAGCGTGGCTCTAACTTCA-3' and reverse 5'-CACAGCATGTTCTTCCCTCA-3'. The primers for rat β -actin were: forward 5'-ACTATCGGCAATGAG CGGTTC-3' and reverse 5'-AGAGCC-ACCAATCCAC ACAGA-3'. The thermal cycle profile for *Lif*, *Lifr* and *gp130* was as follows: denaturation at 94°C for 90 s, ten cycles (denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min) followed by 20 cycles (denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min) and final extension at 72°C for 10 min. The thermal cycle profile for β -actin was as follows: denaturation at 94°C for 90 s, ten cycles (denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min) followed by 18 cycles (denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min) and final extension at 72°C for 10 min. Ten microliters of sample was loaded onto 1.2% agarose gel.

After PCR, all products revealed a single band on agarose electrophoresis. They were purified with PCR Clean Up columns (Qiagen, Hilden, Germany) and fluorescently labelled with the Big Dye Terminator Cycle Sequencing method (Version 1.1) according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA, USA).

Subsequently the products were analysed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) and finally the sequences were identified by an online BLAST search [25].

Real-time PCR analysis

For some experiments, real-time quantitative PCR was used. For this purpose, cDNA was prepared from 500 ng of total RNA following DNase treatment and 10 ng RNA equivalent was used for PCR with specific primers in the presence of SYBR Green I. A melt curve analysis was performed at the end of each reaction. Data (mean $\pm$ SEM) were obtained from four independent experiments. Expression levels were normalised to individual β -actin. The quantity of the specific gene relative to β -actin was

compared between the alloxan condition and the control condition.

Immunohistochemical staining

For immunohistochemical staining of LIF and gp130, pancreatic tissue was embedded in OCT (optimum cutting temperature) compound (Sakura, Zoeterwoude, the Netherlands) and frozen on a mixture of hexane and dry ice. Cryosections (5 μ m) were fixed for 10 min in buffered 4% formaldehyde, and we used the indirect immunostaining method with fluorescein isothiocyanate (FITC)- and tetramethyl rhodamine isothiocyanate (TRITC)-labelled secondary antibodies. A goat anti-LIF antibody (1/100) (N-18; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and an FITC-labelled anti-goat antibody (1/50) (Sigma) were used as first and secondary antibody, respectively, for the staining of LIF. For the staining of gp130 we used a rabbit anti-gp130 antibody (1/100) (C-20; Santa Cruz Biotechnology) and an FITC-labelled anti-rabbit antibody (1/200) (Jackson ImmunoResearch, West Grove, PA, USA). As a control for LIF and gp130 staining, the antibody was preabsorbed with excess of blocking peptide, which resulted in the absence of specific staining. For staining of pancreatic ducts, a mouse anti-cytokeratin-20 (CK20) antibody (1/10) (Novocastra, Newcastle upon Tyne, UK) and a TRITC anti-mouse antibody (1/100) (Jackson ImmunoResearch) or a rabbit anti-polyclonal anti-keratin (PAK) (1/10,000) (Dako, Carpinteria, CA, USA) and an FITC anti-rabbit antibody were used as primary and secondary antibodies respectively. For BrdU staining, 4 μ m paraffin sections were cut and stained with the streptavidin-biotin method and the reaction product was visualised with diaminobenzidine as described [6]. For this staining, antigen retrieval by means of proteinase K treatment (15 μ g/ml for 25 min at 37°C) was performed. A mouse anti-BrdU antibody (1/10) (ICN, Ohio, USA) and a biotinylated anti-mouse antibody (1/300) (Amersham, Roosendaal, The Netherlands) or a TRITC anti-mouse antibody were used. Apoptotic cell death was quantified after propidium iodide staining as described [26].

Western blotting

Protein extraction from pancreas tissue and immunoblotting ($n=3$) were performed as described [27]. The sample (50 μ g) was loaded on the gel. Anti-LIF antibody (1/250) (N-18; Santa Cruz Biotechnology) and horseradish peroxidase-coupled anti-goat antibody (1/10,000) (Santa Cruz Biotechnology) were used as first and secondary antibody, respectively. As a control the antibody was preabsorbed with excess of blocking peptide, which resulted in the absence of the specific band on the blot. For STAT3 western blotting, rabbit antibodies for phosphorylated STAT3 (phospho Y705) and total STAT3 (1/1,000) (Abcam, Cambridge, UK) were used as primary antibodies. As a secondary antibody we used a horseradish peroxidase-coupled anti-

Fig. 1 Double immunohistochemical staining of normal rat pancreas for LIF (a, c), the duct cell marker CK20 (b) and insulin (d). LIF staining is seen in a duct (a, b) but not in an islet (c, d). Bar=20 μ m

rabbit antibody (1/1,000) (Amersham, Uppsala, Sweden). As a control, whole-cell lysates of 3T3-L1 adipocytes with or without stimulation with LIF (Abcam) were loaded. The area and mean density of the bands were measured using the computer program NIH Image 1.63 (<http://rsb.info.nih.gov/nih-image/>).

Statistical analysis

The results are given as mean $\pm$ SEM. Statistical significance was assessed with Student's *t* test for paired data ($p < 0.05$).

Fig. 2 RT-PCR for *Lif*, *Lifr*, *gp130* and β -actin (*Actb*): freshly isolated exocrine cells (lane a), metaplastic exocrine cells after 4 days of culture (lane b), freshly isolated ducts (lane c) and water (lane d)

Fig. 3 Western blot for LIF: metaplastic exocrine cells cultured for 4 days (lane a), freshly isolated exocrine cells (lane b), ductal fluid (lane c)

Results

Expression of LIF in normal and regenerating pancreas

LIF expression was analysed by immunohistochemistry, RT-PCR and western blotting. Analyses were repeated at least three times independently and were found to give very consistent results. Immunostaining on cryosections of the pancreas showed cytoplasmic LIF immunoreactivity exclusively in the larger ducts of the pancreas (Fig. 1a,b), especially close to the luminal surface of the cells (Fig. 1c, d). Expression of *Lif* mRNA was confirmed by RT-PCR in isolated hand-picked duct fragments (Fig. 2). *Lif* mRNA (Fig. 2) and protein (Fig. 3) was also detected in metaplastic exocrine cells after 4 days of culture [23], whereas freshly isolated acinar cells were negative.

Pancreatic tissue after duct ligation consists of ductal complexes [6]. These ductal complexes also exhibit LIF immunoreactivity with staining at the luminal surface of the ducts (Fig. 4). The relatively high amount of ductal cells in the duct-ligated pancreas is probably the reason why *Lif* can only be detected in the duct-ligated part of the pancreas and not in the normal rat pancreas by RT-PCR in total tissue extract (Fig. 5). Only after increasing the number of cycles above 35 could *Lif* also be detected in the normal pancreas (not shown). By western blotting, LIF antiserum detected a band of approximately 43 kDa in rat pancreatic tissue (Fig. 6). We detected a stronger band with a slightly higher molecular weight in the duct-ligated part of the pancreas. This difference in molecular weight of

Fig. 4 Section from duct-ligated pancreas immunostained for LIF (green), CK20 (red) and DNA (blue). Bar=20 μ m

Fig. 5 RT-PCR for *Lif*, *Lifr*, *gp130* and β -actin (*Actb*): rat pancreas with duct ligated for 7 days (lane a), normal rat pancreas (lane b) and water (lane c)

about 2–3 kDa might be due to differences in O-linked oligosaccharides [28].

To find out if ductal pancreatic cells were able to secrete LIF, ductal fluid was collected from the main duct of the pancreas after occlusion of the duct for 2 days. Indeed, the 43 kDa band could be detected in this fluid by western blotting (Fig. 3). This confirms the ability of pancreatic cells to secrete LIF in vivo. Also, metaplastic exocrine cells, but not the freshly isolated exocrine cells, were able to synthesise LIF protein, as shown by western blotting (Fig. 3).

Expression of the receptor complex

We wondered whether the components of the LIF receptor complex were also present in the rat pancreas. The IL6 family of cytokines all use gp130 as a signal-transducing element in different functional receptor complexes [29]. The functional LIF receptor complex consists of the specific LIFR [30] and gp130 [31]. Both *Lifr* and *gp130* could be detected by RT-PCR in rat pancreatic tissue (Fig. 5). Immunostaining on rat pancreatic cryosections showed gp130 immunoreactivity in centro-acinar cells and in ducts, which were identified by CK20 staining (Fig. 7a,b).

Cytospins of freshly isolated rat exocrine pancreatic cells confirmed the gp130 immunoreactivity in the membrane of centro-acinar cells (not shown) and duct fragments

Fig. 6 Western blot for LIF: rat pancreas with duct ligated for 7 days (lanes a, c) and normal rat pancreas (lanes b, d). Antibody (LIF Ab) preabsorbed with peptide was used as negative control (lanes c, d)

Fig. 7 Double immunohistochemical staining of gp130 (a, c) and CK20 (b, d) in normal rat pancreas (a, b) and in duct-ligated rat pancreas (c, d). Bar=40 μ m

(Fig. 8a,b). Cultured exocrine acinar cells, which show features of metaplasia to duct-like cells [23, 32], also showed gp130 staining on the membrane (not shown). The presence of the receptor components in these cells was confirmed by RT-PCR. Freshly isolated exocrine acini and metaplastic exocrine cells expressed both *gp130* and *Lifr*, as was also the case for isolated ducts from the normal rat pancreas (Fig. 2).

When tissue injury and metaplasia were induced by duct ligation, upregulation of LIF and its receptor occurred in rat pancreas (Fig. 5). Gp130 was located in the ductal complexes by immunostaining on frozen sections (Fig. 7c,d).

LIF-induced increase in activation of STAT3

Activation of the LIFR/gp130 complex preferentially induces tyrosine phosphorylation of STAT3. Phosphorylated STAT3 proteins form homodimers and translocate to the

Fig. 8 Double immunostaining of a hand-picked duct fragment for gp130 (a) and the duct cell marker CK20 (b). Bar=20 μ m

Fig. 9 Western blot for STAT3-phospho form (STAT3P) and STAT3: freshly isolated exocrine cells stimulated with LIF (lane a), exocrine cells not stimulated (lane b), 3T3-L1 adipocytes stimulated with LIF (lane c) and 3T3-L1 adipocytes not stimulated (lane d)

nucleus [22]. After incubation of freshly isolated exocrine acini, which still contain centro-acinar cells, with LIF for 15 min there was upregulation of the activated STAT3-phospho form compared with the normal STAT3 level (Fig. 9). There was a mean increase of 1.6 compared with control cells which had not been stimulated with LIF ($p=0.0128$; $n=3$). Stimulation of metaplastic exocrine cells did not lead to any further increase in the expression of the STAT3-phospho form (not shown), probably because these cells already express LIF endogenously.

Stimulation of proliferation of duct cells by LIF

To investigate the possible role of LIF in the normal pancreas, we added LIF for 24 h to a culture of isolated ducts. Proliferation was quantified in sections of PAK-positive duct explants (Fig. 10) after incubation with BrdU for 6 h. We saw a 1.9-fold increase in duct cell proliferation in the LIF condition compared with the control condition ($p=0.015$; $n=3$) (Fig. 11). This shows that LIF is mitogenic for pancreatic duct cells.

Inhibition of proliferation of duct cells by inhibition of JAK2 phosphorylation

Since LIF is produced by the ducts, we wanted to investigate the function of endogenous LIF by inhibiting the JAK/STAT signal transduction, as well as the involvement of this signalling pathway in the effect of exogenous LIF.

Fig. 10 Section from a paraffin-embedded duct explant immunostained for PAK (green), BrdU (red) and DNA (blue). Bar=40 μ m

Fig. 11 Proliferation index ($\pm$ SEM) of duct explants treated or not treated with LIF and AG490 ($n=4$). *Statistically significantly different from control

For this purpose a JAK2 phosphorylation inhibitor [33], AG490, or a combination of AG490 and LIF was added to the culture of isolated duct fragments. After a culture period of 24 h with these factors and labelling for 6 h with BrdU, we found a significant mean decrease in proliferation of 73% in the duct explants of the AG490 condition ($p=0.0078$; $n=4$) and a mean decrease in proliferation of 67% in the AG490+LIF condition compared with the control condition ($p=0.0091$; $n=4$) (Fig. 11).

There was no difference in the number of apoptotic cells in these duct explants, as quantified by propidium iodide staining (control condition, $0.9\pm0.002\%$; AG490 condition, $1.2\pm0.003\%$; AG490 + LIF condition, $1.0\pm0.003\%$). This indicates that the inhibitor was not toxic for the cells at the concentration used.

Upregulation of expression of *Lif* and its receptor components in exocrine cells after alloxan treatment

Cultured exocrine cells (4 days) were incubated with or without alloxan for 1 h, after which RNA was extracted. Quantitative RT-PCR revealed that alloxan upregulated the expression of *Lif*. There was an eight-fold increase in *Lif*

Fig. 12 Quantitative RT-PCR: relative quantity of *Lif* expression ($\pm$ SEM) in exocrine cells with or without alloxan treatment. * $p=0.0053$ ($n=4$)

mRNA expression in the alloxan condition compared with the control condition (relative quantity, 0.058 ± 0.028 for control vs 0.468 ± 0.078 in the alloxan condition; $p=0.0053$; $n=4$) (Fig. 12). There was also a trend towards higher expression of *Lifr* and *gp130*, but this was not significant.

Discussion

We have previously developed experimental models wherein robust beta cell regeneration and expansion can be induced [6–8, 10]. In these particular experimental models we have shown that beta cell replication is not the major mechanism, but rather that new beta cells originate from progenitor cells in the exocrine pancreas. In vitro, the combination of LIF and EGF induces beta-cell neogenesis from beta-cell-depleted primary cultures of metaplastic exocrine cells [10]. Nothing is known about the function of LIF in pancreatic tissue, but in the nervous system LIF is known to act as an injury factor, triggering the early-injury phenotype within neurones and potentially boosting their regenerative potential during secondary nerve repair [34]. Using an immunoassay to determine the LIF content of different organs in neonatal rats, it has been shown that a relatively high level of LIF protein is found in the pancreas, gut, skin, skeletal muscle and lung [35]. As far as we know, the expression and role of LIF in the adult pancreas has not been investigated yet.

In the present study, we show by immunohistochemistry that LIF protein is present in normal ducts and is absent in acini and islets. RT-PCR analysis of isolated duct fragments confirms that *Lif* is expressed at the mRNA level. Furthermore, western blot analysis of ductal fluid shows that LIF protein is secreted into the duct lumen. When acinar exocrine tissue is cultured to allow acinoductal metaplasia [23], LIF becomes expressed by the metaplastic cells.

We also demonstrate the functional gp130 receptor complex for LIF in duct cells and centro-acinar cells by immunohistochemistry and RT-PCR. This suggests a paracrine or autocrine function of LIF. Although an antibody specific for the LIFR did not cross-react with rat tissue, we found immunoreactivity in the ducts of human pancreas biopsies (unpublished observations).

The expression of LIF cytokine and that of its receptor are both increased in the partial duct-ligated pancreas tissue, a model of tissue injury leading to islet regenerative growth from metaplastic exocrine cells [6]. In duct-ligated pancreas, an inflammatory reaction is induced. Since mice receiving a high dose of LIF develop pancreatitis [36], a possible role of LIF in inflammatory reactions in the pancreas can be suggested. Increased expression of LIF is also seen in metaplastic exocrine cells obtained in vitro. We previously showed that in these cells (when cultured as monolayers instead of aggregates) LIF in conjunction with EGF induces beta cell neogenesis [10].

By showing upregulation of the phosphorylated form of STAT3 in our cell preparations after stimulation with LIF, we demonstrate that LIF can activate the receptor and that it

is functional. It is known that in isolated exocrine cells the phosphorylated form of STAT3 can be upregulated after treatment with IFNG [37]. This could play a role in the regenerative events that are induced in the exocrine pancreas of transgenic mice with pancreatic overexpression of IFNG [5].

To get a better understanding of the role of LIF in the pancreas, we stimulated isolated duct explants with exogenous LIF. This resulted in increased duct cell proliferative activity. In transgenic mice ectopically expressing *Lif* under the control of the insulin promoter, increased duct cell proliferation was observed in vivo, which is in accordance with our findings [38]. Thus, auto- or paracrine LIF may stimulate duct cell proliferation under conditions of tissue injury, as in the case of duct ligation, in which increased duct cell proliferation has been noted [6]. Under physiological conditions, we can speculate that LIF controls the proliferation of duct cells, especially in the larger ducts, where there is a higher turnover because of desquamation of the duct-lining cells [39]. It remains to be established whether LIF also plays a role in the development of pancreatic cancers, which are predominantly of the ductal phenotype.

We also blocked the signalling pathway in duct explant cultures by using AG490, an inhibitor of JAK2 activation. It has been shown that blockade of the JAK/STAT pathway inhibits growth of human pancreatic cancer cells [40], certain lines of which express *Lif* [41], but the effect on primary cells has not been investigated. Culture of isolated duct explants with AG490 resulted in strongly diminished proliferation of the cells, suggesting an auto- or paracrine role for LIF in duct cell proliferation. It has also been suggested that the JAK/STAT pathway is important in the regulation of genes related to anti-apoptosis [42]. However, we did not see a difference in apoptotic cell death in the duct cells in the presence or absence of AG490 in the culture medium.

In vivo, we found that gastrin can stimulate or induce beta-cell neogenesis when administered in conjunction with either duct ligation [7] or with alloxan and EGF [8]. Duct ligation is known to stimulate local production of TGFA, a member of the EGF family [43]. In vitro, the combination of EGF with LIF was shown to induce beta-cell neogenesis from exocrine pancreatic cells [10]. It could be that gastrin in vivo might act indirectly, for instance by stimulating local production of LIF in the pancreas. Unfortunately, we could not treat animals with LIF because systemic administration leads to many side effects [36], as is the case for cytokines in general. Another possibility, however, is that gastrin and LIF act via a common signal transduction pathway leading to endocrine cell differentiation of the progenitors. Indeed, it has recently been shown that the CCK2 gastrin receptor in pancreatic cells can activate the JAK2/STAT3 pathway [44]. This pathway is the one which is also activated by LIFR/gp130 signalling [22]. It is also known that EGF can activate this pathway and that the conjunction of EGF and LIF increases the signal over a threshold necessary to achieve major differences in neural cell differentiation [45]. Thus, gastrin/EGF

and LIF/EGF are combinations of neogenesis-inducing agents which can be used for induction of beta-cell regeneration in vivo and in vitro, respectively. Gastrin receptor [32] and LIF receptor (this study) are both expressed by metaplastic exocrine cells, which we believe act as beta-cell progenitors [10]. These agents can stimulate the proliferation of metaplastic exocrine cells [32] and duct cells (this study) respectively. Another interesting observation is that in vitro treatment of metaplastic exocrine cells with alloxan significantly increases the expression of *Lif* by these cells. We have previously noticed that gastrin/EGF treatment induces beta cell expansion (regeneration) in animals previously treated with alloxan, but not in control animals that have not been treated with alloxan [8]. It is thus possible that alloxan treatment in vivo leads to increased LIF production and that it is involved in the induction of beta-cell regeneration in vivo. It must be further investigated whether alloxan exerts its effect on duct cells directly or indirectly via factors released from, for example, damaged beta cells.

In conclusion, our observations show that the LIF cytokine plays a role in normal pancreatic physiology, controls duct cell proliferation and is involved in repair processes following pancreatic tissue injury.

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