Activation and expression of ERK, JNK, and p38 MAP-kinases in isolated islets of Langerhans: implications for cultured islet survival

Steven Paraskevas[a], Reid Aikin[b], Dusica Maysinger[b], Jonathan R.T. Lakey[c], Thomas J. Cavanagh[d], Bernhard Hering[e], Rennian Wang[a], Lawrence Rosenberg[a,*]

[a] *Department of Surgery, The Montreal General Hospital, Montreal, Que., Canada*
[b] *Department of Pharmacology, McGill University and The Montreal General Hospital, Montreal, Que., Canada*
[c] *Medical Surgical Research Institute, University of Alberta, Edmonton, Alta., Canada*
[d] *Roche Molecular Biochemicals, Indianapolis, IN, USA*
[e] *Department of Surgery, University of Minnesota, Minneapolis, MN, USA*

Received 15 June 1999

Abstract Isolation and purification of islet cells exposes them to ischemic, osmotic and mechanical stresses. The objective of this study was to determine the roles of the MAP-kinases in islets immediately following isolation. During the first 48 h, activity of JNK1 and JNK2 declined markedly. Activity of p38 increased steadily with time in culture while extracellular signal regulated kinase (ERK) activity declined dramatically within 24 h post-isolation. High p38 activation relative to ERK activation immediately following isolation correlated with a decrease in islet survival after 36 h in culture. Absence and/or transiency of ERK signaling in conjunction with sustained activation of p38 pathway could be an important regulator of cell death in islets during and following their isolation by commonly employed procedures.

Key words: Islets of Langerhans; Mitogen activated protein kinase; Extracellular signal regulated kinase; JNK; p38; Apoptosis

1. Introduction

The use of cellular grafts of purified islets of Langerhans has provided only transient reversal of hyperglycemia in a small proportion of diabetic recipients [1]. Unfortunately, the primary cause of graft failure is poorly understood. In experimental models of islet transplantation, loss of islet cell mass appears to be a common feature [2,3]. While no reasons have been advanced to explain these changes, we recently reported that human islets undergo apoptosis upon isolation [4], and it has been suggested that this may continue following transplantation as well [5,6]. The extent to which this might represent a response to unfavorable conditions in the extracellular milieu both in vitro and at the graft site is unknown.

The MAP-kinases (MAPK) are serine/threonine kinases which mediate intracellular phosphorylation events triggered by a variety of extracellular stimuli reviewed in [7,8]. These stimuli include growth and differentiation factors, cytokines and integrin-mediated cell attachment. Recently other MAP-kinase subgroups have been identified. Both the JNK (SAPK) family [9–11] and p38 (SAPK2) [12] are activated by various forms of extracellular stress, including cytokines, heat and osmotic shock, endotoxin and UV irradiation [10,11,13–17]. Both JNK and p38 are believed to regulate apoptosis in response to noxious stimuli or growth factor withdrawal in many cell types [18–20].

The extracellular signal regulated kinase (ERK) family of MAPKs regulate intracellular pathways which respond to growth and survival promoting stimuli, including mitogens, survival factors and matrix-integrin interaction [21,22]. It is thought that a crucial balance exists between ERK and the SAPK activity, maintained by the makeup of the cell's microenvironment and ultimately determining the fate of the cell [18,23].

The determinants of this balance in islet cells are not known. Furthermore, the effects of pancreatic dissociation and islet purification on MAPK activation have not been determined. Results from this study show changes in MAPK activation and expression, as well as an increase in expression of the regulatory element MAP-kinase phosphatase-1 (MKP-1) in canine, porcine and human islets following isolation. The trends observed may explain the vulnerability of islet cells to injury and apoptotic cell loss, and may direct efforts at promoting cell survival in the context of transplantation.

2. Materials and methods

2.1. Human islet isolation

Pancreata were retrieved from heart-beating cadaveric donors (age 18–45 years) at the time of multi-organ harvest for transplantation. Consent for donation of tissues for research was obtained by the local organ procurement organization. Following vascular flushing with University of Wisconsin solution perfusion, the pancreas was removed and transported to the islet isolation laboratory. Cold ischemia time was between 30 min and 5 h.

Islets were isolated using the method of Ricordi et al. [24]. Briefly, a 30°C solution of 2 mg/ml Liberase enzyme blend (Roche Molecular, Indianapolis, IN, USA) in Hanks' balanced salt solution (HBSS) (Mediatech, Herndon, VA, USA) supplemented with 0.2 mg/ml DNase I (Boehringer-Mannheim, Montreal, Que., Canada) was infused into the main pancreatic duct using a syringe. The distended pancreas was placed in a sterilized aluminum digestion chamber (Bio-Rep, Miami, FL, USA) through which HBSS supplemented with penicillin (100 000 U/l) and fungizone (2500 μg/l) (Gibco, Burlington, Ont., Canada) was recirculated at 37°C. Extent of tissue digestion was assessed by staining aliquots of digestate with dithizone (Sigma, St. Louis, MO, USA), and visualizing the islets under an inverted light microscope (Nikon, Montreal, Que., Canada). The digestion process was terminated by cooling the circuit to between 5 and 10°C when the majority of islets were free of surrounding acinar tissue. The digestate was collected, centrifuged ($400 \times g$) and washed three times in HBSS-FCS solution and islets were purified on a discontinuous EuroFicoll density gradient (Eurocollins solution and Polysucrose 400, Mediatech, Herndon, VA, USA; Ficoll, Sigma, St. Louis, MO, USA) using a COBE 2991 Cell Processor (COBE BCT, Denver, CO, USA).

*Corresponding author. Fax: (514) 9348210.

PII: S0014-5793(99)00882-0

2.2. Porcine islet isolation

Porcine islets were isolated using Ricordi et al.'s automated method [25] with previously described modifications [26]. Briefly, pancreata were procured from slaughtered sows weighing 200–300 kg, intraductally distended with Liberase HI (Roche Molecular Biochemicals), and digested in a continuous digestion-filtration device at 28–32°C. Free islets were separated from non-islet tissue utilizing isopycnic iodixanol gradients on a COBE 2991 cell separator [27]. Purified islets were cultured free-floating in bicarbonate-free Medium 199 [28] (Sigma, St. Louis, MO, USA) in a humidified atmosphere of 100% air at 37°C for 36 h. Medium 199 was supplemented with 20% (v/v) donor pig serum, 25 mM HEPES (Sigma), and 20 mg/ml ciprofloxacin (Bayer Corporation). Islet quality assessment was performed according to international standards [29].

2.3. Canine islet isolation

Canine pancreata were used to overcome the shortage of human tissue donors and because of the acceptable reliability and reproducibility of the canine islet isolation process. All procedures were in compliance with national animal care standards and were approved by institutional Animal Care Committees. Pancreata were harvested from 2–4 year old mongrel dogs under general anesthesia and sterile conditions using previously defined protocols [30]. Pancreata were placed in ice-cold saline prior to dissection of fatty and connective tissue. Islet isolation and purification were performed in an identical manner as with human islets, using either Liberase CI enzyme blend or Collagenase P (both Roche Molecular, Indianapolis, IN), Collagenase type 11 (Sigma, St. Louis, MO, USA) or Serva collagenase (Crescent Chemical, Hauppauge, NY, USA).

2.4. Culture of islets

Islets were cultured in suspension in CMRL 1066 (Gibco, Burlington, Ont., Canada) with or without supplementation of 10% fetal bovine serum (Wisent, St. Bruno, Que., Canada). Islets isolated in Indianapolis were shipped overnight in serum supplemented CMRL 1066. On arrival, islet equivalent number was reassessed, and culture medium was changed before incubation at 37°C in a humidified atmosphere of 5% CO_2.

2.5. Sample preparation

All islets were removed from the dish and washed twice in cold PBS. The islets were centrifuged and the pellet resuspended in a Nonidet-P40 lysis buffer (Nonidet-P40, PMSF, aprotinin, sodium orthovanadate, Sigma-Aldrich Canada, Oakville, Ont., Canada). The cells were sonicated for 15 s and centrifuged at 14 000 rpm for 20 min. The supernatant was recovered and frozen at −20°C. Minor variations in sample size were corrected by performing a total protein assay on an aliquot of each whole cell lysate using Bradford protein dye (Bio-Rad, Mississauga, Ont., Canada).

2.6. Western blotting

Whole cell lysates were combined with SDS-Laemmli sample buffer (Bio-Rad, Mississauga, Ont., Canada) and electrophoresed on 12% Tris-glycine gels (Novex, Toronto, Ont., Canada) at 100 V. Proteins were transferred to nitrocellulose (Bio-Rad, Mississauga, Ont., Canada) in a Mini-Trans Blot apparatus (Bio-Rad, Mississauga, Ont., Canada) at 250 mA constant current for 90 min. Blots were blocked in 1% bovine serum albumin and 1% ovalbumin (Sigma-Aldrich Canada, Oakville, Ont., Canada) in Tris-buffered saline with Tween 20 (Sigma-Aldrich Canada, Oakville, Ont., Canada) for 60 min at room temperature or overnight at 4°C. Primary antibody solution was added for 60 min at room temperature or overnight at 4°C. All primary antibodies were from Santa Cruz (Santa Cruz, CA, USA) except the following: anti-active ERK and p38 (Promega, Madison, WI, USA) anti-phospho-JNK and anti-phospho-p38 (New England Biolabs, Beverly, MA, USA) and were used as recommended by the manufacturer. Secondary antibody was anti-rabbit IgG conjugated to horseradish peroxidase (Amersham, Oakville, Ont., Canada) and exposure was 60 min at room temperature. The blots were washed in Tris- buffered saline with Tween 20, and bands visualized by enhanced chemiluminescence using ECL reagent and Hyperfilm ECL (Amersham, Oakville, Ont., Canada).

2.7. Blot analysis

All experiments were conducted a minimum of three times and representative blots were analyzed using Gel-Cypher (Light Tools Research) software for Windows.

3. Results

It is difficult to quantify islet cell viability following collagenase digestion and to predict islet survival [31]. We attempted to identify possible biochemical markers to use as predictors for this purpose. MAP-kinase signaling pathways have been implicated in survival and death (apoptosis) signaling in response to numerous stimuli in a variety of cell types. Though the roles of the MAP-kinases may vary somewhat in different cell types, their purpose remains the same: to transmit signals to the nucleus in response to changing environmental conditions. In our case, we attempted to clarify the role of ERK, JNK, and p38 in islets of Langerhans in response to a notoriously variable and unpredictable isolation procedure to gain an understanding of the cellular state of the islet.

The first question we addressed was whether the expression of the MAP-kinases was comparable in different species. ERK, JNK and p38 were readily identifiable in preparations of islets from canine, porcine and human pancreata, using identical protocols and commercially available antibodies (Fig. 1). MAPK expression over time was then examined. Islets cultured in CMRL 1066 with 10% fetal bovine serum maintained constant levels of ERK expression from 0 to 7 days after isolation. (Fig. 2A) JNK 1 (p46) expression was constant throughout the culture period. Expression of JNK 2 (p54), however, increased with time (Fig. 2B). Expression of p38 was found to be constant throughout the 7 day culture period (Fig. 2C). Expression of MKP-1 increased progressively throughout the culture period (Fig. 2D).

MAPK activation was observed in order to compare the extent of signaling through each of the three pathways in response to islet isolation. The activation patterns were found to be similar between species. ERK activity was highest on the day of isolation, and declined after day 1. Both ERK 1 and 2 showed the same pattern of activation, and both showed a modest increase of activity by day 7 (Fig. 3A). The highest levels of JNK 1 and JNK 2 activity were observed on day 0, the day of isolation. Although considerable variation existed between isolations, the temporal pattern that followed was the same. The activity of both JNK 1 and JNK 2 kinases decreased during the first 3 days in culture, and remained low

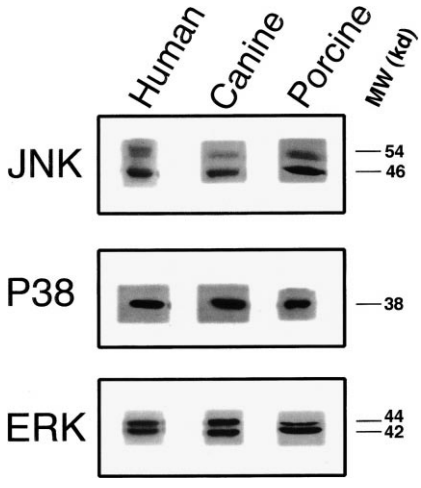

Fig. 1. Expression of MAP-kinases in human, canine, and porcine islets.

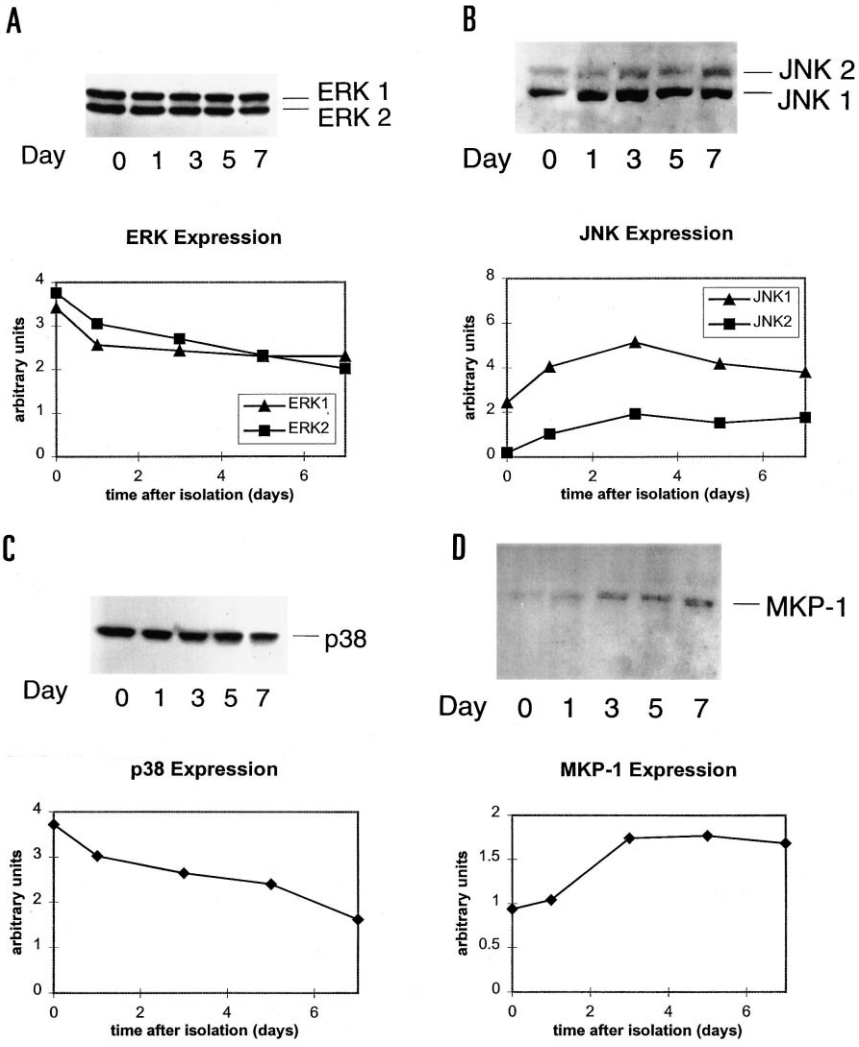

Fig. 2. Expression of MAP-kinases and MKP-1 in canine islets. A: Expression of ERKs. B: Expression of JNKs. C: Expression of p38. D: Expression of MKP-1.

thereafter (Fig. 3B). In contrast to JNK, p38 activity was minimal on the day of isolation, and increased progressively thereafter (Fig. 3C). We compared the degree of phosphorylation of p38 to its activity using a commercially available non-radioactive kinase assay in order to establish a correlation between the two (data not shown).

In comparing the activation of ERK, JNK, and p38 of two separate porcine islet isolations, high p38 activation relative to that of ERK immediately after isolation was observed to correlate with low islet survival following 36 h in culture (Fig. 4). Isolation 1 had higher ERK activation and considerably lower p38 activation when compared to isolation 2. The survival after a 36 h culture period of islets from isolation 1 (115.5% IEQ recovery) was substantially higher than that of isolation 2 (47% IEQ recovery) in which the ERK activation was at the detection limit but p38 activity was higher. Thus, at least partial contribution to the significant difference in survival of these isolations could be ascribed to the striking imbalance of p38 vs. ERK activity.

Results from these studies suggest that p38 and ERK phosphorylation (activation) could provide a diagnostic indicator of islet cell damage and along with other parameters, be used to produce a 'score' of islet viability. According to this, different isolation environments could be tested. Enhanced p38 activity is not the sole parameter associated with diminished islet cell survival, but along with suppressed ERK activity, it is more reliable than either of the JNK kinases. The observed variability of JNK activation in islets cells coupled with our pilot studies using ERK and p38 inhibitors (PD98059 and PD169316) support such a notion.

4. Discussion

Enzymatic and mechanical dissociation of the pancreatic stroma represents a unique hurdle in islet cell transplantation. The exact nature of the injury sustained by the endocrine pancreatic tissue during dissociation and purification has never been adequately assessed. Furthermore, the assumption that purified islets need only a restoration of adequate blood supply to continue to function normally has not been evaluated. The disruption of normal cell-matrix and cell-cell interactions in islets may have dire implications on cell survival.

The injury sustained by islet cells during isolation and transplantation involves several etiologies, including hypoxic, mechanical, osmotic and thermal stress. After transplantation, the hypoxic insult continues until revascularization of the

graft is complete. During and after this period, the cells are subjected to both non-specific inflammatory and alloimmune responses by host defenses.

The tightly regulated pathways controlling cell survival, which are crucial during cell and tissue differentiation, may continue to regulate cell homeostasis both in vivo and in vitro. These pathways are known to include the MAP-kinase signaling cascades [23]. Under homeostatic conditions, there is thought to be balanced signaling through the MAP-kinase pathways in which the ERKs generally mediate growth and survival and the JNK/p38 kinases are associated with death (apoptosis). There is increasing evidence for overlapping roles of the MAP-kinases in particular cases due to the fact that they can share common upstream activators and activate common downstream targets in a variety of cell types (a discussion of which is beyond the scope of this paper, and is reviewed in [7,8,23,32]). However, it has recently been shown that both JNK and p38 avoid cleavage by caspases during apoptosis while ERK is targeted for caspase-dependent proteolysis, again indicating a possible overlying role for p38 and JNK in promoting apoptosis [33].

This study was undertaken in order to elucidate the roles of the MAP-kinases in signaling events leading to apoptosis in isolated islets. These studies therefore examined the expression and activation of the principal MAP-kinases in freshly isolated islets and the changes observed during short-term culture. Similar results were obtained for each of the three species studied, indicating the conservation of these signal transduction elements in higher mammals. The commercially available antibodies were able to label the target proteins such that the same blotting protocol could be used, again indicative of structural conservation.

The ERKs were found to have maximal activity on the day of isolation. This was followed by a period of relative hypoactivity, from which there is partial recovery, by day 7. This decline in ERK activity may have an effect on islet cell death, which has been shown to increase during this period. The expression of ERK, however, was constant throughout the period of observation.

JNK expression differed for the two isoforms examined. JNK 1 expression was relatively constant throughout the study, while JNK 2 was observed to increase steadily over 7 days. This suggests the existence of discrete regulatory processes for these two isoforms at the level of gene expression. Activity of JNK 1 and 2 was highest on day 0, and declined thereafter. The level of activity was constant after day 3. The high levels observed may represent a response to the stress of isolation. The high activation of JNK in this context could result in a wave of apoptotic cell death occurring within days of isolation. This wave of apoptotic activity has been observed in both human [4], and canine islets (data not shown). The increasing level of JNK 2 expression in the face of low levels of activity could signify an increasing sensitivity of the JNK pathway to stimuli. This result also suggests that there is no redundancy between isoforms. A third band was sometimes observed but it was unclear whether it was due to a shift in electrophoretic migration due to phosphorylation or if this band represents JNK 3.

The p38 MAP-kinase is thought to participate in the cell stress response in a manner similar to JNK, but differing in the target transcription factors. The p38 kinase has been shown to mediate the death of some cell types by apoptosis as a result of withdrawal of growth factors [18,20] and cell culturing [19]. In addition, some of the downstream targets of p38 have been implicated in stress response. The recently characterized kinase PRAK is one such target which is acti-

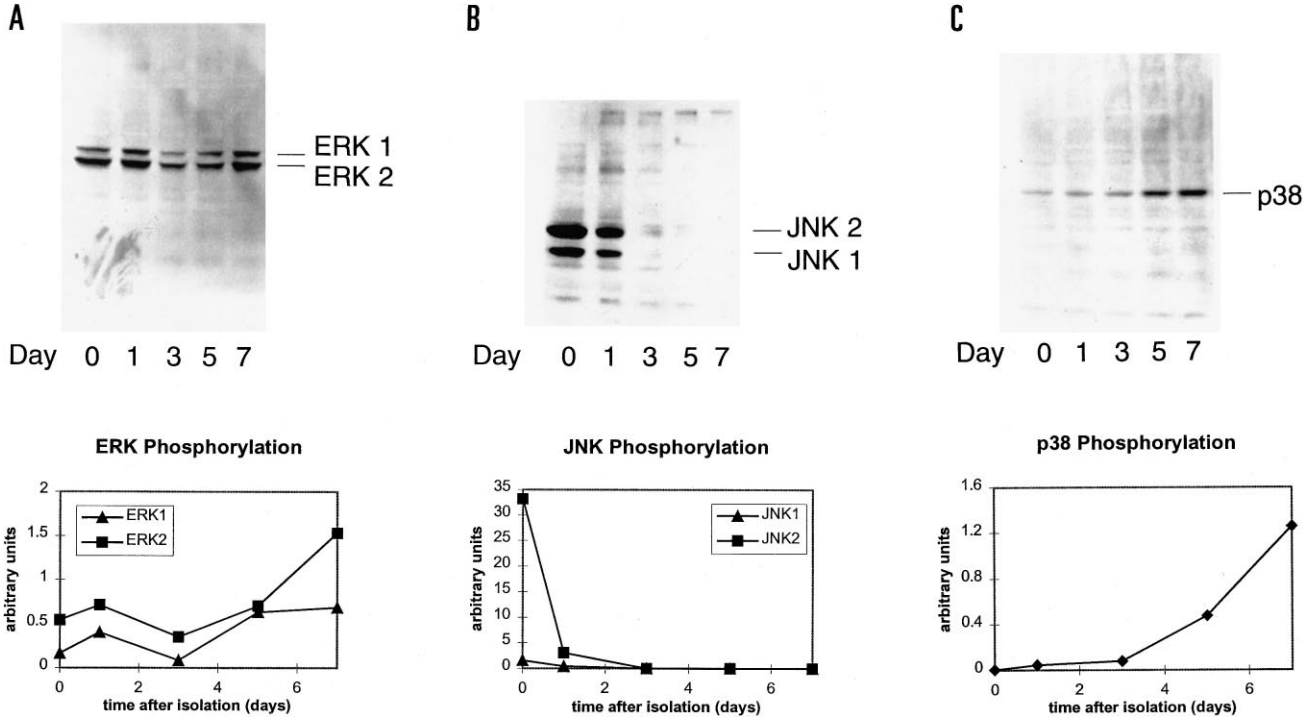

Fig. 3. Phosphorylation (activation) of MAP-kinases in canine islets. A: Phosphorylation of ERKs. B: Phosphorylation of JNKs. C: Phosphorylation of p38.

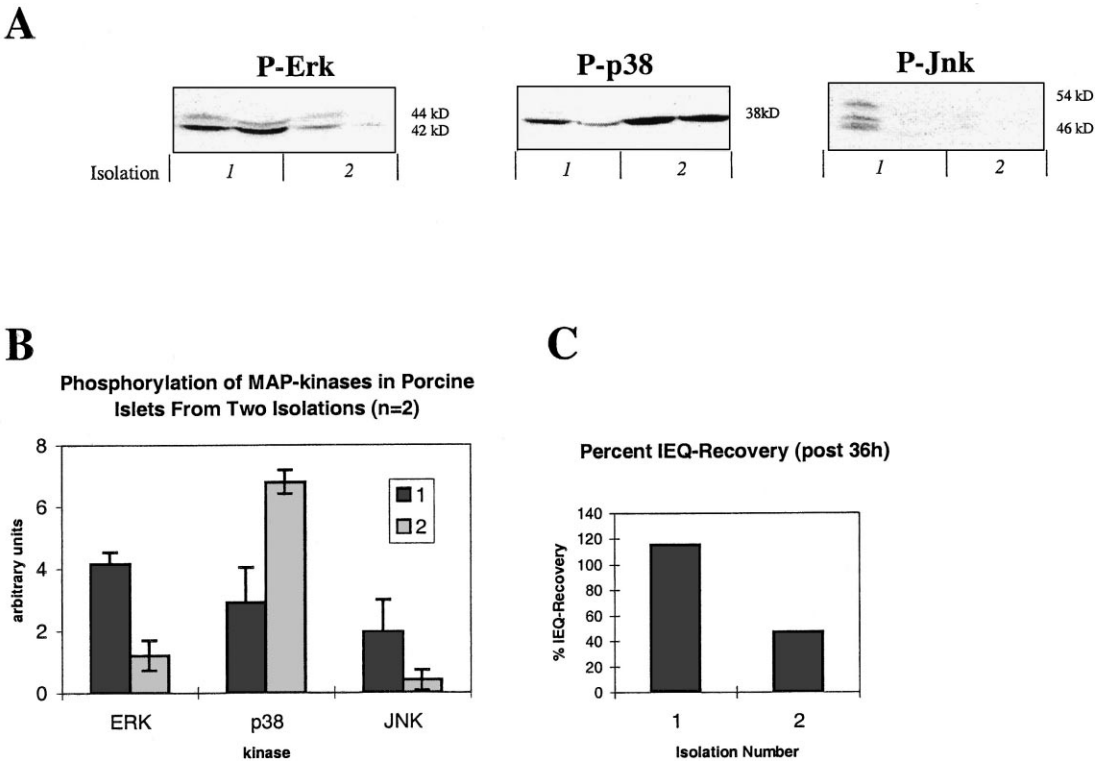

Fig. 4. Phosphorylation of ERK, JNK, and p38 and survival in porcine islets immediately following isolation. A: Western blots for phosphorylation of the kinases. B: The means of the relative intensity of kinases phosphorylation ± S.E.M. C: Islet survival after 36 h culture.

vated in response to stress [34]. In most of the islets studied however, p38 activity was not appreciable on the day of isolation. Instead, it appeared to increase gradually with time. There is limited evidence for a physiological role for p38, and it has been observed in only a few types of cells. In hepatocytes, a constant, constitutive level of p38 expression exists, and this activity declines in response to stress [35]. In islets, p38 is thought to mediate the transcription of insulin via the insulin upstream factor-1 in response to the hyperglycemic stimulus [36]. This physiologic response is known to be inhibited by cytotoxic stress. It is possible that the low levels of p38 activity are a response to the high stress of isolation, and the gradual rise represents a recovery of the viable cells from this insult. Clearly, the p38 response differs remarkably from that of JNK, and this alone represents a distinction between the role of p38 in primary islet cells and transformed cell lines.

The opposite roles of ERK and p38 on islet cell survival demonstrated here in porcine islets are consistent with previous studies on PC12 cells in which the cells were stimulated to undergo apoptosis by withdrawal of nerve growth factor [18]. In our case, however, the stimulus leading to apoptosis is the isolation/culturing process itself. This type of spontaneous apoptosis is also demonstrated in cultured human neutrophils, but p38 was not shown to be involved [19]. In the case of porcine isolation 1, there was initially low p38 and high ERK relative to porcine isolation 2. Since isolation 1 showed significantly higher survival after 36 h. culture, it appears that initial ERK and p38 activation after isolation mediate islet cell survival in similar manner to PC12 cells [18]. Based on these results we could not yet draw any definite conclusions about the effect of JNK activity on cell viability in porcine islets. However, it is conceivable that the role of JNK is to counteract the disrupted homeostasis between ERK and p38. If so, then it would follow that indeed JNK activity varies from one isolation to the next depending on the degree of disrupted balance between the two other MAP-kinases.

MKP-1 is one of several isoforms which play an important role in the regulation of cellular activities by MAP-kinases [37]. MKP-1 is induced by a variety of stimuli, including both mitogenic and cytotoxic mediators. In particular, MKP-1 is induced by JNK activity, and may play an important role in the regulation of this and other signals [35]. MKP-1 was increasingly expressed with time. How this response relates to the rising levels of JNK 2, the decline in activity of ERK and the degree of isolation injury, remains to be further clarified.

The high level of JNK activity and rising JNK 2 expression seen after islet isolation and purification, combined with a reduction in ERK activity may represent a potent death signal for the cells in response to this injury. The loss of ERK activity may also represent an adverse change in the cell's microenvironment, with the loss of important trophic signals required for cell survival. The heightened JNK 2 expression could also prove hazardous to cells exposed to a second injury, either ischemic or inflammatory, during subsequent transplantation. The rise in p38 activity could also promote apoptosis, but the responses of p38 and JNK appear to differ fundamentally in islet cells.

Clearly, cell loss prior to engraftment is a significant barrier to the success of islet transplantation, as the majority of clinical islet transplants never demonstrate any sustained graft function [1]. Isolation and purification of islets exposes them to mechanical, osmotic and ischemic stresses, the precise consequences of which have been poorly characterized. In addi-

tion, isolation may remove important cell-cell and cell-matrix interactions contributing to maintenance of the cell population in the intact pancreas. The roles of the MAPKs in islet cells have not been extensively studied, and no studies have been published about their responses in isolation, purification and transplantation. They are however, clearly linked to the processes of apoptosis and survival, and the ability to manipulate them may prove to be important, if not crucial, to the maintenance of a functioning islet cell graft. Further studies will focus on whether the balance of MAPK activity can be altered in favor of growth promotion and survival.

Acknowledgements: The work was supported (D.M. and L.R.) by the Canadian Diabetes Association. S.P. was supported by a fellowship from the Canadian Diabetes Association. J.R.T.L. was supported by a post-doctoral fellowship from the Alberta Heritage Foundation for Medical Research

References

[1] Hering, B., Schultz, A., Geier, C., Bretzel, R. and Federlin, K. (1995) Islet Transpl. Regist. Newslett. 6, 1–27.
[2] Alejandro, R., Cutfield, R., Shienvold, F., Polonsky, K., Noel, J., Olson, L., Dillberger, J., Miller, J. and Mintz, D. (1986) J. Clin. Invest. 78, 1339–1348.
[3] Lanza, R.P., Sullivan, S.J. and Chick, W.L. (1992) Diabetes 41 (12), 1503–1510.
[4] Paraskevas, S., Duguid, W.P., Maysinger, D., Feldman, L., Agapitos, D. and Rosenberg, L. (1997) Transplant. Proc. 29, 750–752.
[5] Davalli, A., Scaglia, L., Zangen, D., Hollister, J., Bonner-Weir, S. and Weir, G. (1995) Transplant. Proc. 27, 3238–3239.
[6] Davalli, A., Scaglia, L., Zangen, D., Hollister, J., Bonner-Weir, S. and Weir, G. (1996) Diabetes 45, 1161–1167.
[7] Force, T. and Bonventre, J.V. (1998) Hypertension 31, 152–161.
[8] Karin, M. (1998) Ann. NY Acad. Sci. 139–145.
[9] Kyriakis, J.M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E.A., Ahmad, M.F., Avruch, J. and Woodgett, J.R. (1994) Nature 369, 156–160.
[10] Han, J., Lee, J.D., Bibbs, L. and Ulevitch, R.J. (1994) Science 265, 808–811.
[11] Derijard, B., Hibi, M., Wu, I.H., Barrett, T., Su, B., Deng, T., Karin, M. and Davis, R.J. (1994) Cell 76, 1025–1037.
[12] Wang, Z., Harkins, P.C., Ulevitch, R.J., Han, J., Cobb, M.H. and Goldsmith, E.J. (1997) Proc. Natl. Acad. Sci. USA 94, 2327–2332.
[13] Raingeaud, J., Gupta, S., Rogers, J.S., Dickens, M., Han, J., Ulevitch, R.J. and Davis, R.J. (1995) J. Biol. Chem. 270, 7420–7426.
[14] Lo, Y.Y.C., Wong, J.M.S. and Cruz, T.F. (1996) J. Biol. Chem. 271, 15703–15707.
[15] Kyriakis, J.M. and Avruch, J. (1996) BioEssays 18, 567–577.
[16] Hambleton, J., Weinstein, S.L., Lem, L. and DeFranco, A.L. (1996) Proc. Natl. Acad. Sci. USA 93, 2774–2778.
[17] Chen, Y.R., Wang, X., Templeton, D., Davis, R.J. and Tan, T.H. (1996) J. Biol. Chem. 271, 31929–31936.
[18] Xia, Z., Dickens, M., Raingeaud, J., Davis, R.J. and Greenberg, M.E. (1995) Science 270, 1326–1331.
[19] Frasch, S.C., Nick, J.A., Fadok, V.A., Bratton, D.L., Worthen, G.S. and Henson, P.M. (1998) J. Biol. Chem. 273, 8389–8397.
[20] Krummer, J.L., Rao, P.K. and Heidenreich, K.A. (1997) J. Biol. Chem. 272, 20490–20494.
[21] Miyamoto, S., Teramoto, H., Gutkind, J.S. and Yamada, K.M. (1996) J. Cell Biol. 135, 1633–1642.
[22] Zhu, X. and Assoian, R.K. (1995) Mol. Biol. Cell 6, 273–282.
[23] Jarpe, M.B., Widmann, C., Knall, C., Schlesinger, T.K., Gibson, S., Toshiaki, Y., Fanger, G.R., Gelfand, E.W. and Johnson, G.L. (1998) Oncogene 17, 1475–1482.
[24] Ricordi, C., Lacy, P., Finke, E., Olack, B. and Scharp, D. (1988) Diabetes 37 (4), 413–420.
[25] Ricordi, C., Socci, C., Davalli, A.M., Staudacher, C., Pierangelo, B., Vertova, A., Sassi, I., Gavazzi, F., Pozza, G. and Di Carlo, V. (1990) Surgery 107, 688–694.
[26] Brandhorst, H., Brandhorst, D., Hering, B.J. and Bretzel, R.G. (1999) Transplantation (in press).
[27] Lake, S.P., Bassett, P.D. and Larkins, A. et al. (1989) Diabetes 38, 143.
[28] Davalli, A.M., Bertuzzi, F. and Socci, C. et al. (1993) Transplantation 56, 148–154.
[29] Ricordi, C., Gray, D.W.R. and Hering, B.J. et al. (1990) Acta Diabetol. Lat. 27, 185–195.
[30] Lakey, J.R., Cavangh, T.J., Zieger, M.A.J. and Wright, M.J. (1998) Cell Transplant. 7, 365–372.
[31] London, N.J.M., Swift, S.M. and Clayton, H.A. (1998) Diabetes Metab. 23, 200–207.
[32] Paul, A., Wilson, S., Belham, C.M., Robinson, C.J.M., Scott, P.H., Gould, G.W. and Plevin, R. (1997) Cell. Signal. 9, 403–410.
[33] Widmann, C., Gibson, S. and Johnson, G.L. (1998) J. Biol. Chem. 273, 7141–7147.
[34] New, L., Jiang, Y., Zhao, M., Liu, K., Whu, W., Flood, L.J., Kato, Y., Parry, G.C.N. and Han, J. (1998) EMBO J. 17, 3372–3384.
[35] Mendelson, K.G., Contois, L.R., Tevosian, S.G., Davis, R.J. and Paulson, K.E. (1996) Proc. Natl. Acad. Sci. USA 93, 12908–12913.
[36] Macfarlane, W.M., Smith, S.B., James, R.F., Clifton, A.D., Doza, Y.N., Cohen, P. and Docherty, K. (1997) J. Biol. Chem. 272, 20936–20944.
[37] Chu, Y., Solski, P.A., Khosravi-Far, R., Der, C.J. and Kelly, K. (1996) J. Biol. Chem. 271, 6497–6591.