ELSEVIER

Biochemical Pharmacology 61 (2001) 1561–1569

Biochemical Pharmacology

Role of p38 mitogen-activated protein kinase (p38 MAPK) in cytokine-induced rat islet cell apoptosis

Johan Saldeen[a], John C. Lee[b], Nils Welsh[a,*]

[a]*Department of Medical Cell Biology, Uppsla University, Biomedicum, P.O. Box 571, S-751 23 Uppsala, Sweden*
[b]*SmithKline Beecham Pharmaceuticals, King of Prussia, PA, USA*

Received 27 July 2000; accepted 1 November 2000

Abstract

The signaling pathways mediating nitric oxide production and apoptosis in pancreatic β-cells are not fully understood. We investigated cytokine-induced protein phosphorylation events in insulin-producing cells and evaluated their role in inducible nitric oxide synthase (iNOS) induction and cell death. Interleukin-1β (IL-1β), but not interferon-γ (IFN-γ), induced phosphorylation of p38 mitogen-activated protein kinase, c-Jun NH2-terminal kinase, and mitogen- and stress-activated protein kinase 1 (MSK1) in rat insulin-producing RINm5F cells. This was paralleled by an increased phosphorylation of the transcription factors activating transcription factor-2 (ATF-2) and cAMP-responsive element-binding protein (CREB). The p38 inhibitor SB203580 prevented cytokine-induced phosphorylation of CREB and MSK1, but not of ATF-2. IFN-γ induced the phosphorylation of signal transducer and activator of transcription 1. The combination of IL-1β and IFN-γ increased both apoptosis and necrosis in rat islet cells. SB203580, but not the extracellular signal-regulated kinase inhibitor PD98059, partially prevented cytokine-induced apoptosis, an effect that was not associated with reduced nitrite production or lowered iNOS expression. In conclusion, cytokine-induced p38 activation participates in β-cell apoptosis, possibly by a nitric oxide-independent mechanism or by enhancing the sensitivity to nitric oxide.

Keywords: Pancreatic islet; p38 MAPK; Apoptosis; Nitric oxide; Interleukin-1; MSK1

1. Introduction

It has been demonstrated that the cytokines IL-1β, TNF-α, and IFN-γ exert inhibitory and cytotoxic effects on rodent pancreatic β-cells *in vitro* [1–3]. This has led to the suggestion that cytokines, alone or in combination, may be important mediators of the autoimmune destruction of β-cells during the course of insulin-dependent diabetes mellitus [2]. Exposure of isolated rat islets *in vitro* to IL-1β and IFN-γ leads to both apoptosis and necrosis [3], and this effect is thought to be mediated, at least in part, by induction of iNOS [3–7]. Nitric oxide production leads to inhibition of aconitase, glucose oxidation rates, ATP generation, and insulin production *in vitro* [4–7]. Moreover, it has recently been shown that transgenic mice overexpressing iNOS in insulin-producing cells develop diabetes, and that this was associated with DNA fragmentation and β-cell destruction [8]. The deleterious effects of IL-1β are clearly potentiated by IFN-γ [9]. It seems that IFN-γ augments IL-1β-induced nitric oxide production, which could explain the enhanced β-cell death observed with this specific combination of cytokines [9].

Cytokine-mediated signal transduction in insulin-producing cells seems to involve pathways leading to activation of JNK, p38 MAPK (p38), ERK, and STAT1 [10–12]. JNK and p38 belong to the MAPK family and are, as opposed to the classic MAP kinases ERK1 and ERK2, preferentially stimulated in response to stress and specific cytokines. Exposure of cells to stress or proinflammatory cytokines modulates the activities of intracellular signals such as IL-1 receptor-associated kinase (IRAK) [13], TNF receptor-associated factor 2 (TRAF2) [14], ceramide [15], or GTP-binding proteins [16] that promote activation of MAP kinase/ERK kinase kinase (MEKK) and possibly also MEKK

* Corresponding author. Tel.: +46-18-471.4212; fax: +46-18-556.401.

E-mail address: Nils.Welsh@medcellbiol.uu.se (N. Welsh).

Abbreviations: IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; CREB, cAMP-responsive element-binding protein; iNOS, inducible nitric oxide synthase; ATF-2, activating transcription factor-2; STAT, signal transducer and activator of transcription; and MSK, mitogen- and stress-activated protein kinase.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00605-0

upstream kinases [17]. We have previously observed that IL-1β activates JNK1 in the insulin-producing cell line RINm5F [10]. This event was paralleled by an increased c-Jun and ATF-2 phosphorylation and an enhanced binding activity to the ATF/CREB consensus element [10]. Furthermore, IL-1β has been shown to stimulate the phosphorylation of the transcription factor Elk-1 and heat shock protein 25 [11]. The precise role of these pathways in IL-1β-induced gene expression and cell death is currently unknown. In other cell systems, prolonged stimulation of p38 or JNK appears to promote apoptosis, whereas ERK activation seems to promote cell survival [18]. In view of these findings, the aim of the present investigation was to study the putative involvement of cytokine-induced phosphorylation events in the signaling leading to β-cell death. Special emphasis was given to the roles of p38 and ERK in cytokine-induced islet cell apoptosis and necrosis.

2. Materials and methods

2.1. Reagents

[4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole] (SB203580) was synthesized at Smith-Kline Beecham. PD98059 (2′-amino-3′-methoxyflavone) was from Calbiochem-Novabiochem Corporation. Human recombinant IL-1β was provided by Dr. K. Bendtzen of the Laboratory of Medical Immunology, Rigshospitalet, Copenhagen, Denmark. The cytokine was produced by Immunex and had a biological activity of 50 U/ng, as compared with an interim international standard rIL-1β preparation (NIBSC, London, UK) [19]. Recombinant mouse IFN-γ was from AMS Biotechnology Ltd. Recombinant murine TNF-α was from R&D Systems.

2.2. RINm5F cell and pancreatic islet culture

Growing RINm5F cells (passage number >120) were trypsinized every 3–5 days and subcultured (1×10^5 cells per each 10-mm well or 5×10^5 cells per each 50-mm well) in RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, benzylpenicillin (100 U/mL), and streptomycin (0.1 mg/mL) at 37° in humidified air with 5% CO_2.

Rat islets were isolated from male Sprague–Dawley rats (local Uppsala colony) and mouse islets from NRMI mice (B&K Universal) as described [20]. Islets were precultured for 2–5 days under the conditions described above before incubation with cytokines and inhibitors.

2.3. Immunoblot analysis

RINm5F cells ($2–3 \times 10^5$) or mouse islets (groups of 75) were exposed to cytokines (20 min) and SB203580 (20 + 20 min) as stated in the figure legends. Cells were washed in cold PBS and directly lysed in SDS–β-mercaptoethanol sample buffer containing 1 mM phenylmethylsulfonyl fluoride. Samples were then run on 7.5–12% SDS–polyacrylamide gels and electroblotted to nitrocellulose filters. The filters were then incubated with anti-ATF-2 (C-19) antibodies (Santa Cruz Biotechnology, Inc.), phospho-specific (Ser133) CREB antibodies, phospho-specific (Thr183/Tyr185) JNK antibodies, phospho-specific (Thr180/Tyr182) and phospho-independent p38 MAPK antibodies, phospho-specific (Thr202/Tyr204) MAP kinase antibody, phospho-specific (Tyr701) Stat1 antibody, and phospho-specific MSK1 (Thr581) antibody (all from New England Biolabs) diluted 1:400–1000 in Tris-buffered saline (TBS) supplemented with 2.5% bovine serum albumin. Horseradish peroxidase-linked goat anti-rabbit immunoglobin was used as a second layer. The immunodetection was performed as described for the enhanced chemiluminescence (ECL) immunoblotting detection system (Amersham International). Following the ECL procedure, filters were stained for 2 min in 100 μg/mL of amido black in 10% methanol + 5% acetic acid. The filters were then repeatedly washed for 5 min with 10% methanol + 5% acetic acid and then allowed to air dry. The intensities of the enhanced ECL bands and prominent amido black bands were quantified by densitometric scanning using Kodak Digital Science 1D software (Eastman Kodak).

For detection of iNOS protein levels in isolated rat islets, precultured islets in groups of 100 were cultured overnight in the presence of 25 U/mL of IL-1β + 1000 U/mL of IFN-γ with or without SB203580 (10 μM) or PD98059 (10 μM). The islets were lysed in SDS–β-mercaptoethanol buffer and separated on 7% SDS–polyacrylamide gels. Nitrocellulose filters were immunoblotted with a mouse monoclonal NOS2 (C-11) antibody (Santa Cruz Biotechnology).

2.4. Nitrite determination

To isolated rat islets, in groups of 100, different substances were added as given in the figures. The next day, duplicate samples (2×80 μL) were taken for nitrite determination as previously described [6].

2.5. Detection of apoptosis/necrosis by flow cytometry

Islets were cultured in the presence of cytokines or inhibitors for 24 hr. A previously described method was used for quantification of apoptosis and necrosis [21]. This method discriminates between apoptosis and necrosis by the ability of propidium iodide to freely enter necrotic cells, whereas the uptake of propidium iodide into apoptotic cells is only slightly increased as compared to viable cells [21]. Briefly, islets were incubated for 15 min with propidium iodide (10 μg/mL), rinsed in PBS, and subsequently trypsinized for 8 min at 37°. By incubating the islets with

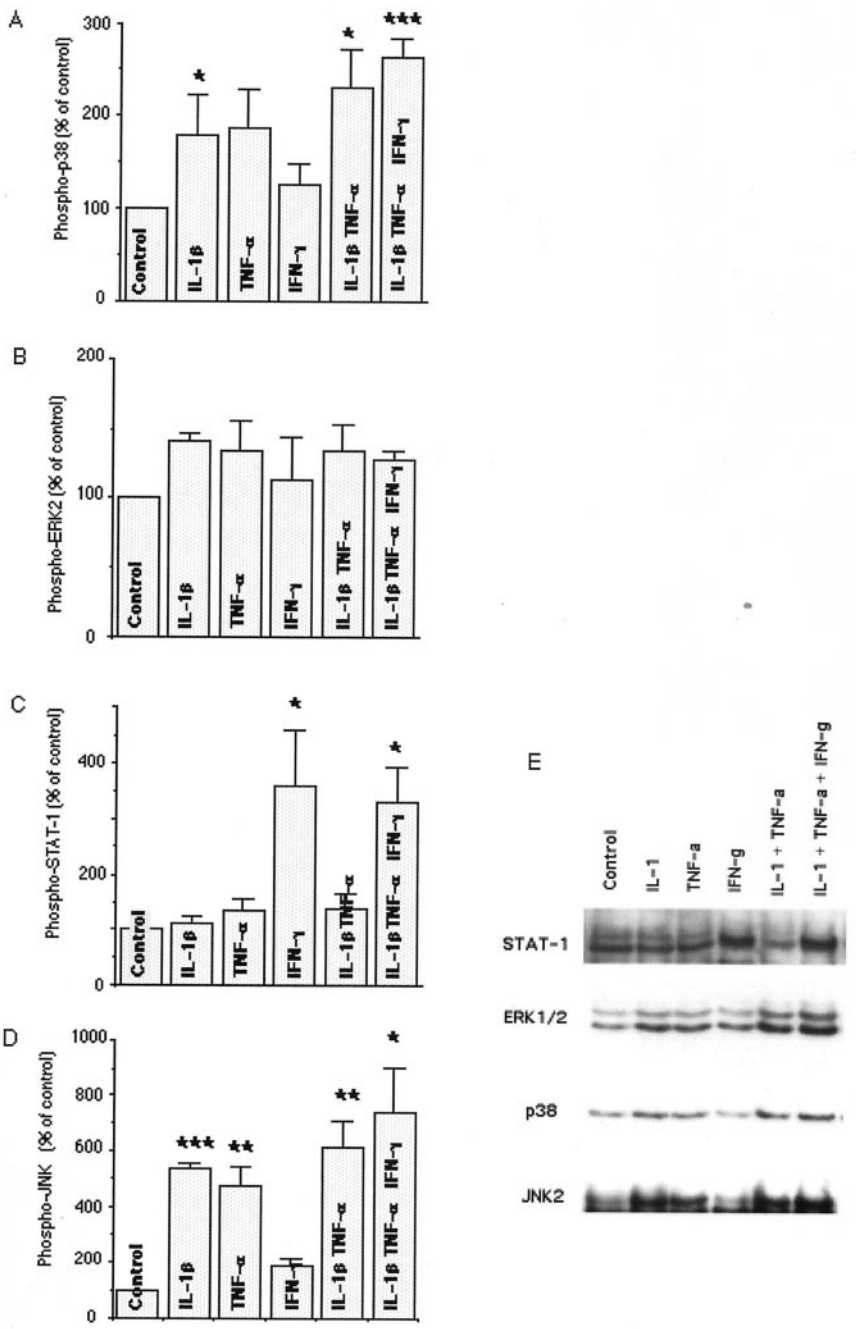

Fig. 1. Effects of IL-1β, TNF-α, and IFN-γ on phosphorylation of p38, ERK2, STAT1, and JNK2. RINm5F cells were exposed for 20 min to IL-1β (25 U/mL), TNF-α, (1000 U/mL), IFN-γ (1000 U/mL), or combinations of cytokines as given in the figure. This was followed by determination of p38, ERK2, STAT1, and JNK2 phosphorylation. Total protein loading onto the gels was assessed by amido black staining of the nitrocellulose filters. Results from densitometric scanning of phospho-p38, phospho-ERK2, phospho-STAT1, and phospho-JNK2 were normalized to total protein loading, as assessed by amido black staining, and expressed as percent of control. Results are means ± SEM for three observations. *, **, and *** denote $P < 0.05$, 0.01, and 0.001, respectively.

propidium iodide prior to the trypsination procedure, trypsination artifacts are avoided. The dispersed islet cells were then washed and resuspended in culture medium containing fetal calf serum. The cells were analyzed in a Becton Dickinson FACSCalibur flow cytometer with respect to their forward scatter and FL_3 fluorescence. Data were analyzed using the CellQuest software (Becton Dickinson Instruments).

2.6. Statistical analysis

Data are presented as means ± SEM. Comparisons were made by one-way ANOVA and Student's *t*-test.

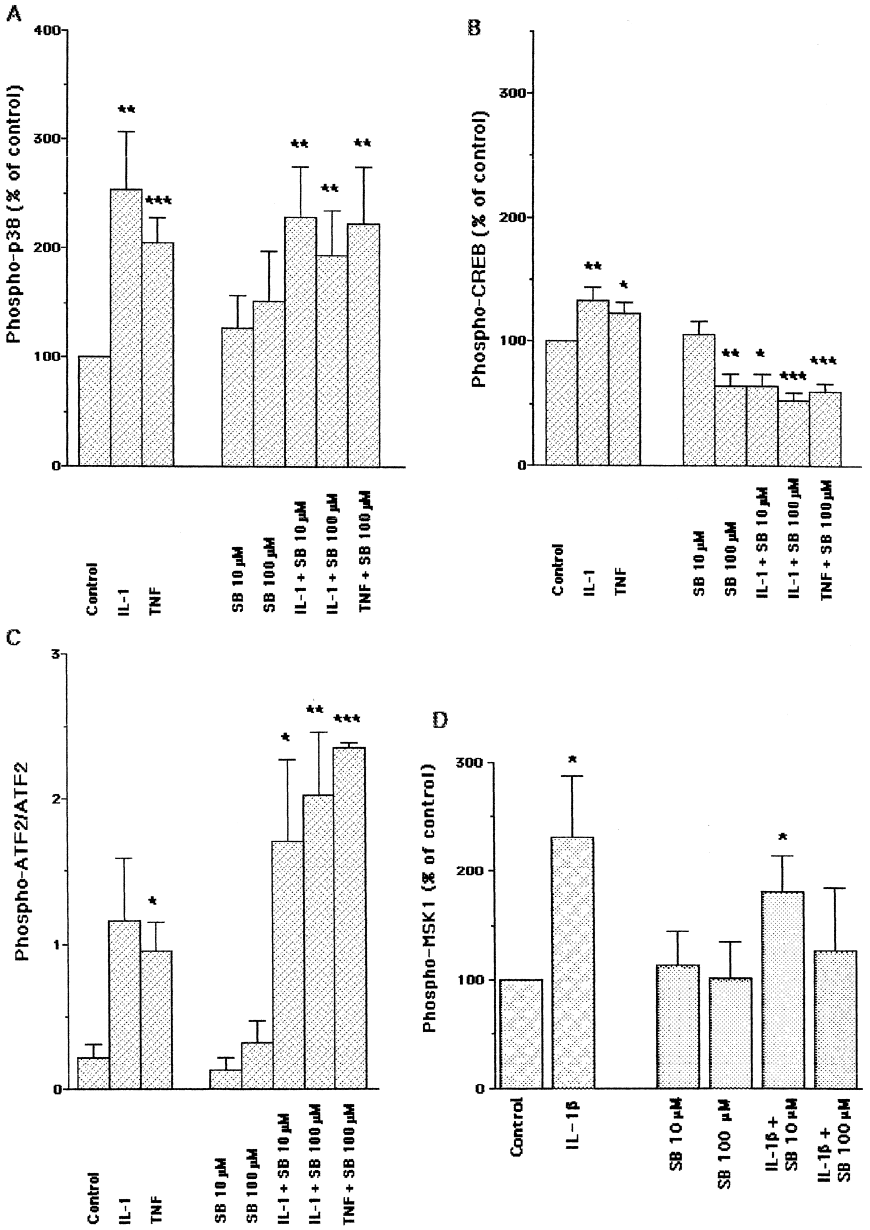

Fig. 2. Effects of IL-1β, TNF-α, and SB203580 on ATF-2, CREB, p38, and MSK1 phosphorylation. RINm5F cells were exposed for 20 min to IL-1β (25 U/mL), TNF-α (1000 U/mL) and/or 10 or 100 μM SB203580 as given in the figure followed by determination of ATF-2 (A), CREB (B), p38 (C), or MSK1 (D) phosphorylation. SB203580 was added 20 min before cytokines. Results are means ± SEM for 3 (A), 4 (B), 3–7 (C), or 3 (D) observations. *, **, and *** denote $P < 0.05$, 0.01, and 0.001, respectively. The phospho-CREB and phospho-38 signals were expressed per non-phosphorylated p38 detected on the same filters. Phospho-ATF-2 was expressed per the lower non-phosphorylated ATF-2 band. In panels E and F, typical ATF-2, phospho-CREB, phospho-p38, p38, and phospho-MSK1 bands are shown.

3. Results

3.1. Effects of IL-1β, TNF-α, and IFN-γ on phosphorylation of p38, ERK, STAT-1, and JNK2

Exposure of mouse pancreatic islets for 20 min to 25 U/mL of IL-1β resulted in increased phosphorylation of ERK2, P38, and JNK2 (338 ± 76, 230 ± 16, and 751 ± 169 percent of control, respectively. $P < 0.05$ versus control using Student's paired *t*-test, N = 3), as assessed by immunoblotting of cell extracts with phospho-specific antibodies. A 20-min exposure of RINm5F cells to IL-1β (25 U/mL) or TNF-α (1000 U/mL) resulted in an approximate 2-fold increase in the phosphorylation of p38 and a 5-fold increase in JNK2 phosphorylation (Figs. 1 and 2). JNK1 phosphorylation was affected in a similar manner as that of JNK2 (results not shown). Thus, there is a good correlation between IL-1β-induced phosphorylation of p38 and JNK in primary islet cells and that observed in RINm5F cells. However, there was only a non-significant trend to a higher

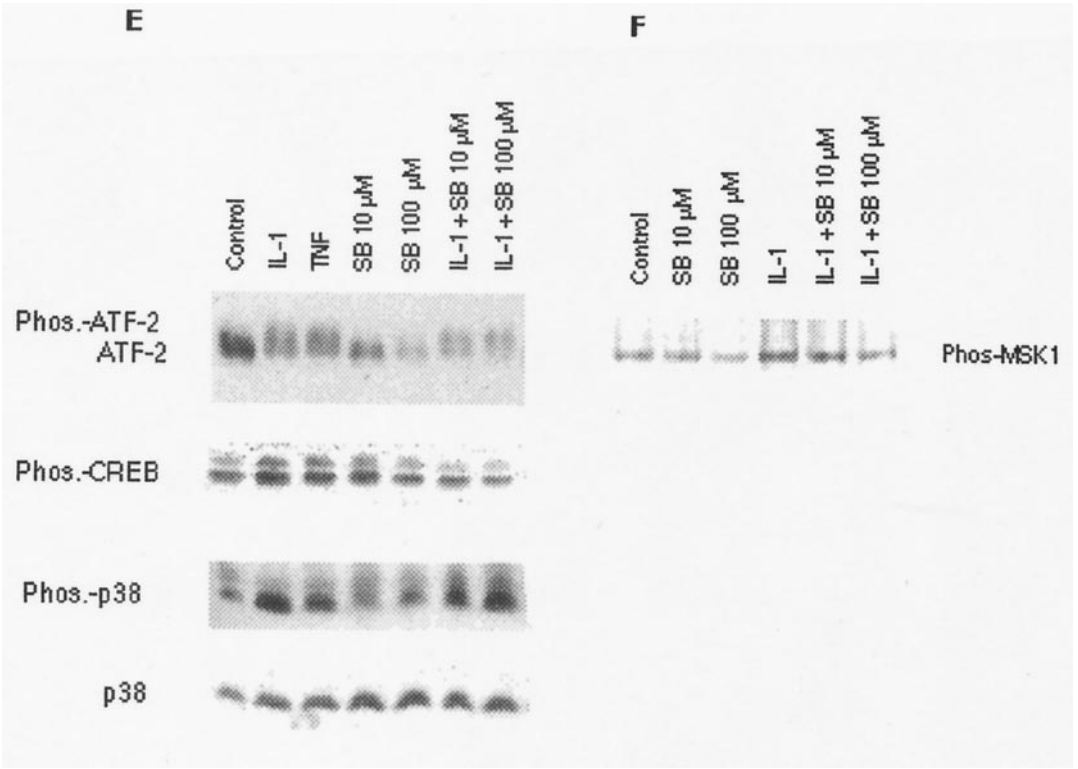

Fig. 2. (*continued*)

ERK2 phosphorylation in response to IL-1β or TNF-α (Fig. 1). This discrepancy could possibly be due to the fact that RINm5F cells are rapidly proliferating with a high basal ERK activity, which is not easily stimulated further. In view of the similar JNK and p38 responses to IL-1β in islet and RINm5F cells, the insulinoma cell line was used for subsequent phosphorylation studies. There was no clear additive effect when combining IL-1β and TNF-α (Fig. 1), indicating that the two cytokines might act by stimulating the same pathways. IFN-γ (1000 U/mL) did not stimulate p38, ERK, or JNK phosphorylation, either by itself or when combined with IL-1β and TNF-α (Fig. 1). However, IFN-γ increased the phosphorylation of STAT1 by a factor of 3–4 (Fig. 1).

To reveal the role of p38 in different β-cell signal transduction pathways, phosphorylation of p38 and the p38 substrates MSK1, CREB, and ATF-2 was studied. A 20-min exposure of RINm5F cells to IL-1β or TNF-α evoked phosphorylation of p38, which was paralleled by a modest increase in phosphorylation of the transcription factor CREB (Fig. 2). IL-1β also promoted a 2-fold increase in the phosphorylation of MSK1 (Fig. 2). The transcription factor ATF-2 is separated into two bands by SDS–PAGE, the lower representing unphosphorylated ATF-2 and the higher phosphorylated ATF-2 [10]. Both IL-1β and TNF-α induced a shift from the unphosphorylated to the phosphorylated form (Fig. 2).

Addition of the specific p38 inhibitor SB203580 (10 μM) alone did not affect the basal phosphorylation of ATF-2, MSK1, or p38 (Fig. 2). However, when 10 μM of SB203580 was added 20 min before the cytokines, IL-1β- and TNF-α-induced phosphorylation of CREB was prevented without affecting the phosphorylation of p38 itself (Fig. 2). Similar results were obtained with the higher concentration of SB203580 (100 μM). SB203580 is known to inhibit p38 activity without preventing p38 phosphorylation [22]. On the other hand, phosphorylation of the p38 substrate MSK1 in response to IL-1β was only partially prevented by 10 μM SB203580 (Fig. 2). It was only at the higher concentration (100 μM) that SB203580 completely abolished the IL-1β effect. Interestingly, SB203580, at either concentration, did not prevent IL-1β- or TNF-α-induced phosphorylation of ATF-2. These results indicate that CREB is mainly phosphorylated by p38, MSK1 by p38 and other cytokine-activated kinases, and ATF-2 mainly by JNK.

3.2. Effects of SB203580 or PD98059 on cytokine-induced islet cell apoptosis/necrosis

To study the putative roles of p38 or ERK1/2 in apoptosis of islet cells, rat islets were cultured overnight in the presence of SB203580 (10 μM) or PD98059 (10 μM) with or without the combination of IL-1β and IFN-γ. The combination of cytokines increased the fractions of cells with intermediate (apoptotic cells) and strong fluorescence (necrotic cells) (Fig. 3). The increase in apoptosis was partially prevented by SB203580, but not by PD980579 (Fig. 3).

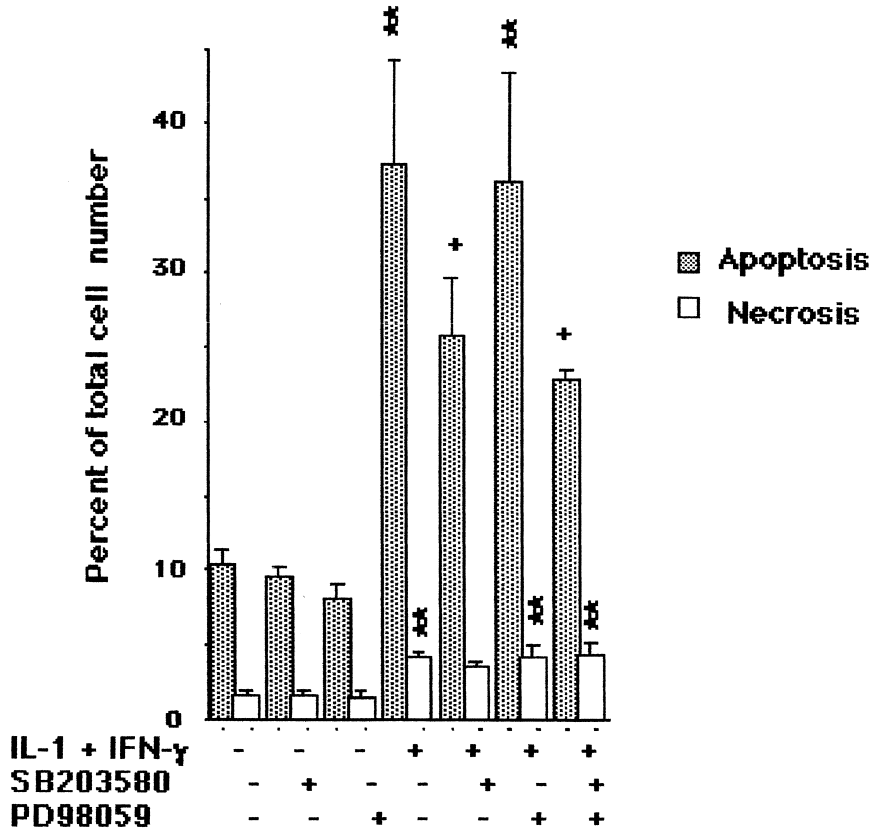

Fig. 3. IL-1β- and IFN-γ-induced islet cell apoptosis is counteracted by SB203580. Isolated rat islets were cultured overnight in the presence or absence of IL-1β (25 U/mL) and IFN-γ (1000 U/mL). To some groups of islets were added 10 μM SB203580 or 10 μM PD98059. The number of cells with intermediate (apoptosis) or high (necrosis) propidium iodide uptake was determined using flow cytometry. A total of 10,000 cells were counted in each group. Results are means ± SEM for 6 separate observations. ** denotes $P < 0.01$ when comparing versus control islet cells using one-way ANOVA and Dunnett's test. $^{+}$ denotes $P < 0.05$ versus cytokine-exposed islet cells using Student–Newman–Keul's test.

SB203580 or PD98059 did not affect islet cell apoptosis or necrosis when added in the absence of cytokines.

3.3. Effects of SB203580 or PD98059 on cytokine-induced nitric oxide production and iNOS protein levels in isolated rat islets

An overnight incubation period in the presence of IL-1β and IFN-γ resulted in high levels of nitrite (Fig. 4). This was paralleled by a strong induction of a band with iNOS immunoreactivity (Fig. 5). SB203580 affected neither nitrite production nor iNOS protein expression in response to the combination of IL-1β and IFN-γ. Interestingly, the nitrite levels were somewhat higher in response to cytokines and PD98059 than to cytokines alone (Fig. 4). This was not paralleled by an increase in the iNOS protein expression (Fig. 5). Nitrite levels from control islets were not above background.

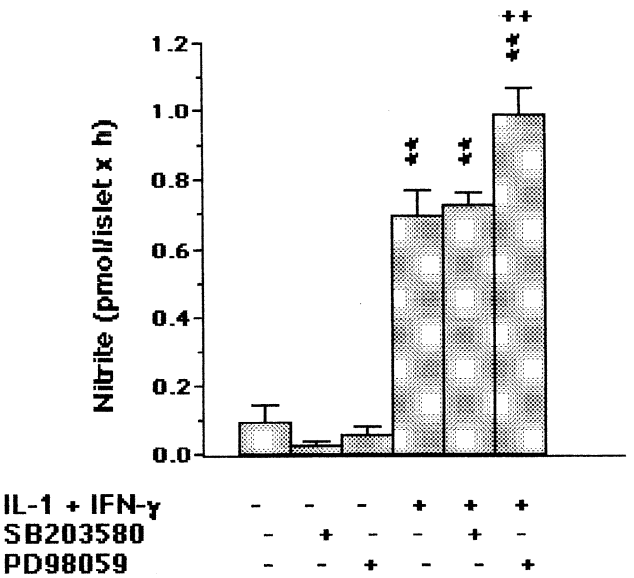

Fig. 4. Effects of SB203580 or PD98059 on IL-1β- and IFN-γ-induced islet nitrite production. Isolated rat islets were exposed overnight to the additions given in the figure and formation of nitrite was determined. Results are means ± SEM for 3–6 observations. ** denotes $P < 0.01$ versus control islets (Dunnett's test), $^{++}$ denotes $P < 0.01$ versus cytokine-treated islets (Tukey's test).

4. Discussion

We have demonstrated that IL-1β promotes phosphorylation of p38 and JNK2 to a similar degree in both mouse

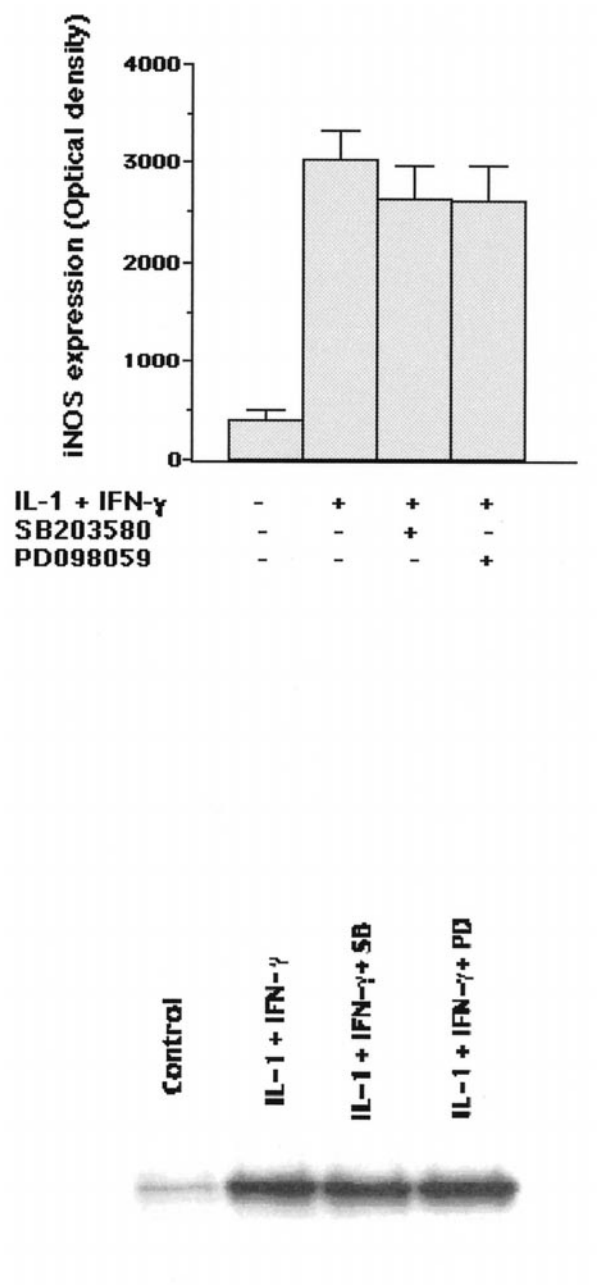

Fig. 5. Effects of SB203580 and PD98059 on IL-1β- and IFN-γ-induced iNOS protein levels. Isolated rat islets were exposed overnight to the additions given in the figure and immunoblot analysis of iNOS was performed. (A) Densitometric analysis of iNOS immunoblots. Results are means ± SEM for 5 observations. (B) Scanned image of a representative immunoblot showing the 120-kDa iNOS band.

islets and RINm5F cells. This suggests that the insulinoma cell line is an appropriate model for the primary islet β-cell in this particular experimental system. The IL-1β-induced increase in p38 and JNK2 phosphorylation conforms to previous studies showing that IL-1β activates JNK1 in RINm5F cells [10] and p38 in neonatal rat islets and RIN-5AH cells [11]. Our finding that TNF-α also stimulated p38 and JNK2 phosphorylation in insulin-producing cells has, to our knowledge, not previously been reported. Since we have previously observed that TNF-α by itself does not induce iNOS gene expression or β-cell apoptosis [3,23], it is likely that activation of p38 and JNK is not a sufficient event for iNOS induction. Also novel is the finding that IL-1β promotes phosphorylation of MSK1 in insulin-producing cells. MSK1 is structurally related to the p90 ribosomal S6 kinase (RSK) and is known to be phosphorylated, in other cell types, by p38 as well as by ERK [24]. Upon activation, MSK1 phosphorylates CREB, ATF-1, and possibly also phospholipase A2 [24,25]. MSK1-induced transcription factor activation may mediate increased cyclooxygenase-2, IL-1β, or early growth response gene-1 (egr-1) gene transcription [26,27]. The presently observed IL-1β-induced MSK1 phosphorylation seems to be, at least in part, mediated by p38, since it was counteracted by SB203580. However, it is unclear whether MSK1 participates in IL-1β-induced iNOS expression or islet cell apoptosis.

The cytokine IFN-γ did not affect the phosphorylation of p38, ERK, or JNK. Nevertheless, IFN-γ is known to potentiate IL-1β-induced nitric oxide production and β-cell death [9,11], an effect possibly related to the presently observed STAT1 activation. Indeed, binding sites for STAT homo- and heterodimers have been observed in the enhancer region of the *iNOS* gene [28].

Because a 24-hr exposure to IL-1β, TNF-α, or IFN-γ alone did not induce significant β-cell apoptosis [3,23], we presently used the combination of IL-1β and IFN-γ to provoke islet cell death. Using the p38 inhibitor SB203580 at the lower concentration (10 μM) on isolated rat islets, we observed a partial protection against IL-1β- and IFN-γ-induced islet cell apoptosis. This is in line with numerous publications supporting a proapoptotic role of sustained p38 activation in β-cells [29] and other cell systems [30–34]. In neonatal rat islets, SB203580 has been reported to decrease IL-1β-induced nitric oxide production and inhibit iNOS mRNA gene expression [11]. This is seemingly contradictory to the present results, which demonstrate that neither nitrite nor iNOS protein levels are decreased by SB203580. However, it may be that iNOS induction by the combined action of both IL-1β and IFN-γ (present results) is less sensitive to p38 inhibition than iNOS induction by IL-1β alone [11]. Nevertheless, the findings of this study favor the view that cytokine-induced p38 activation enhances islet cell sensitivity to nitric oxide or promotes apoptosis by a nitric oxide-independent pathway rather than increasing nitric oxide production. This is in line with the recent observations that cytokines induce apoptosis, but not necrosis, of islet cells from iNOS-deficient mice [35], or that nitric oxide-induced apoptosis in HL-60 cells is mediated by p38 activation [36]. Indeed, inhibition of p38 resulted presently mainly in protection against apoptosis, but not necrosis. Possible pathways downstream of p38 activation are, for example, those leading to caspase activation [37], FasL expression [38], or p53 phosphorylation [39]. The ERK inhibitor PD98059 affected neither apoptosis nor iNOS pro-

tein levels induced by cytokines. This is consistent with the general view that ERK activation leads to cell survival rather than cell death [18]. Surprisingly, however, the nitrite production was slightly enhanced in response to ERK inhibition. Assuming that nitrite levels accurately reflect nitric oxide generation, it is possible that posttranslational control of iNOS is modified by ERK. Whether it could be phosphorylation events or substrate/cofactor availability that mediates this effect is unknown. The beneficial effect of p38 inhibition by SB203580 against cytokine-induced islet cell apoptosis was only partial in the present study, which suggests that other pathways contribute significantly to β-cell death. In addition to the other stress-activated kinases JNK and MSK1, it may be that Ca^{2+} entry [40], ceramide generation [23], or an insufficient Bcl-2 activity [3] mediates cytokine-induced β-cell killing.

In summary, this study is to our knowledge the first to demonstrate a direct role of p38 in cytokine-induced β-cell apoptosis. Moreover, we also show that p38 is not involved in IL-1β-+ IFN-γ-induced iNOS expression. The elucidation of these issues might help us understand the pathogenesis of type 1 diabetes and promote pharmacological treatments that intervene in the autoimmune destruction of β-cells.

Acknowledgments

The excellent technical assistance of Ing-Marie Mörsare and Ing-Britt Hallgren is gratefully acknowledged. This work was supported by grants from the Swedish Medical Research Council (12X-109, 12X-11564, 72P-12995), the Swedish Diabetes Association, the Nordic Insulin Fund, the Juvenile Diabetes Foundation International, and the Family Ernfors Fund.

References

[1] Eizirik DL, Flodström M, Karlsen EA, Welsh N. The harmony of the spheres: inducible nitric oxide synthase and related genes in pancreatic β cells. Diabetologia 1996;39:875–90.
[2] Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA, Svenson M. Cytotoxicity of human pI 7 interleukin-1 for pancreatic islets of Langerhans. Science 1986;232:1545–7.
[3] Saldeen J. Cytokines induce both necrosis and apoptosis via a common Bcl-2-inhabitable pathway in rat insulin-producing cells. Endocrinology 2000;141:2003–10.
[4] Nerup J, Mandrup-Poulsen T, Mølvig J, Helqvist S, Wogensen L, Egeberg J. Mechanisms of pancreatic β-cell destruction in type I diabetes. Diabetes Care 1988;11(Suppl 1):16–23.
[5] Southern C, Schulster D, Green IC. Inhibition of insulin secretion by interleukin-1β and tumour necrosis factor-α via an L-arginine-dependent nitric oxide generating mechanism. FEBS Lett 1990;276:42–4.
[6] Welsh N, Eizirik DL, Bendtzen K, Sandler S. Interleukin-1β-induced nitric oxide production in isolated rat pancreatic islets requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitase. Endocrinology 1991;129:3167–73.
[7] Corbett JA, Wang JL, Hughes JH, Wolf BA, Sweetland MA, Lancaster JR Jr, McDaniel ML. Nitric oxide and cyclic GMP formation induced by interleukin 1β in islets of Langerhans. Evidence for an effector role of nitric oxide in islet dysfunction. Biochem J 1992;287:229–35.
[8] Takamura T, Kato I, Kimura N, Nakazawa T, Yonekura H, Takasawa S, Okamoto H. Transgenic mice overexpressing type 2 nitric-oxide synthase in pancreatic β cells develop insulin-dependent diabetes without insulitis. J Biol Chem 1998;273:2493–6.
[9] Heitmeier MR, Scarim AL, Corbett JA. Interferon-γ increases the sensitivity of islets of Langerhans for inducible nitric-oxide synthase expression induced by interleukin 1. J Biol Chem 1997;272:13697–704.
[10] Welsh N. Interleukin-1 β-induced ceramide and diacylglycerol generation may lead to activation of the c-Jun NH2-terminal kinase and the transcription factor ATF2 in the insulin-producing cell line RINm5F. J Biol Chem 1996;271:8307–12.
[11] Larsen CM, Wadt KA, Juhl LF, Andersen HU, Karlsen AE, Su MS, Seedorf K, Shapiro L, Dinarello CA, Mandrup-Poulsen T. Interleukin-1beta-induced rat pancreatic islet nitric oxide synthesis requires both the p38 and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases. J Biol Chem 1998;273:15294–300.
[12] Heitmeier MR, Scarim AL, Corbett JA. Prolonged STAT1 activation is associated with interferon-γ priming for interleukin-1-induced inducible nitric-oxide synthase expression by islets of Langerhans. J Biol Chem 1999;274:29266–73.
[13] Martin MU, Falk W. The interleukin-1 receptor complex and interleukin-1 signal transduction. Eur Cytokine Netw 1997;8:5–17.
[14] Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF-kappaB induction by TNF, CD95 and IL-1. Nature 1997;385:540–4.
[15] Westwick JK, Bielawska AE, Dbaibo G, Hannun YA, Brenner DA. Ceramide activates the stress-activated protein kinases. J Biol Chem 1995;270:22689–92.
[16] Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, Xu N, Miki T, Gutkind JS. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell 1995;81:1137–46.
[17] Shi CS, Kehrl JH. Activation of stress-activated protein kinase/c-Jun N-terminal kinase, but not NF-κB, by the tumor necrosis factor (TNF) receptor 1 through a TNF receptor-associated factor 2- and germinal center kinase related-dependent pathway. J Biol Chem 1997;272:32102–7.
[18] Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;270:1326–31.
[19] Svenson M, Bendtzen K. Inhibitor of interleukin 1 in normal human urine. Different effects on mouse thymocytes and on a murine T-cell line. Scand J Immunol 1988;27:593–9.
[20] Andersson A. Isolated mouse pancreatic islets in culture: effects of serum and different culture media on the insulin production of the islets. Diabetologia 1978;14:397–404.
[21] Hortelano S, López-Collazo E, Boscá L. Protective effect of cyclosporin A and FK506 from nitric oxide-dependent apoptosis in activated macrophages. Br J Pharmacol 1999;126:1139–46.
[22] Young PR, McLaughlin MM, Kumar S, Kassis S, Doyle ML, McNulty D, Gallagher TF, Fisher S, McDonnell PC, Carr SA, Huddleston MJ, Seibel G, Porter TG, Livi GP, Adams JL, Lee JC. Pyridinyl imidazole inhibitors of p38 mitogen-activated protein kinase bind in the ATP site. J Biol Chem 1997;272:12116–21.
[23] Saldeen J, Jaffrézzou J-P, Welsh N. The acid sphingomyelinase inhibitor SR33557 counteracts TNF-α-mediated potentiation of IL-1β-induced NF-κB activation in the insulin-producing cell line RINm5F. Autoimmunity 2000;32:241–54.
[24] Deak M, Clifton AD, Lucocq LM, Alessi DR. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. EMBO J 1998;17:4426–41.

[25] Hefner Y, Borsch-Haubold AG, Murakami M, Wilde JI, Pasquet S, Schieltz D, Ghomashchi F, Yates JR 3rd, Armstrong CG, Paterson A, Cohen P, Fukunaga R, Hunter T, Kudo I, Watson SP, Gelb MH. Serine 727 phosphorylation and activation of cytosolic phospholipase A2 by MNK1-related protein kinases. J Biol Chem 2000;275:37542–51.

[26] Caivano M, Cohen P. Role of mitogen-activated protein kinase cascades in mediating lipopolysaccharide-simulated induction of cyclooxygenase-2 and IL-1 beta in RAW264 macrophages. J Immunol 2000;164:3018–25.

[27] Rolli M, Kotlyarov A, Sakamoto KM, Gaestel M, Neininger A. Stress-induced simulation of early growth response gene-1 by p38/stress-activated protein kinase 2 is mediated by a cAMP-responsive promoter element in a MAPKAP kinase 2-independent manner. J Biol Chem 1999;274:19559–64.

[28] Lowenstein CJ, Alley EW, Raval P, Snowman AM, Snyder SH, Russell SW, Murphy WJ. Macrophage nitric oxide synthase gene: two upstream regions mediate induction by IFN-γ and lipopolysaccharide. Proc Natl Acad Sci USA 1993;90:9730–4.

[29] Rosenberg L, Wang R, Parskevas S, Maysinger D. Structural and functional changes resulting from islet isolation lead to islet cell death. Surgery 1999;126:393–8.

[30] Shan R, Price JO, Gaarde WA, Monia BP, Krantz SB, Zhao ZJ. Distinct roles of JNKs/p38 MAP kinase and ERKs in apoptosis and survival of HCD-57 cells induced by withdrawal or addition of erythropoietin. Blood 1999;94:4067–76.

[31] Oh-hashi K, Maruyama W, Yi H, Takahashi T, Naoi M, Isobe K. Mitogen-activated protein kinase pathway mediates peroxynitrite-induced apoptosis in human dopaminergic neroplastoma SH-SY5Y cells. Biochem Biophys Res Commun 1999;263:504–9.

[32] Hida H, Nagano S, Takeda M, Soliven B. Regulation of mitogen-activated protein kinases by sphingolipid products in oligodendrocytes. J Neurosci 1999;19:7458–67.

[33] Birkenkamp KU, Kokter WH, Esselink MT, Jonk LJ, Kruijer W, Vellenga E. A dual function for p38 MAP kinase in hematopoietic cells: involvement in apoptosis and cell activation. Leukemia 1999;13:1037–45.

[34] Aoshiba K, Yasui S, Nishimura K, Nagai A. Thiol depletion induces apoptosis in cultured lung fibroblasts. Am J Respir Cell Mol Biol 1999;21:54–64.

[35] Liu D, Pavlovic D, Chen M-C, Flodström M, Sandler S, Eizirik DL. Cytokines induce apoptosis in β-cells isolated from mice lacking the inducible isoform of nitric oxide synthase (iNOS$-/-$). Diabetes 2000;49:1116–22.

[36] Jun CD, Pae HO, Kwak HJ, Yoo JC, Choi BM, Oh CD, Chun JS, Paik SG, Park YH, Chung HT. Modulation of nitric oxide-induced apoptotic death of HL-60 cells by protein kinase C and protein kinase A through mitogen-activated protein kinases and CPP32-like protease pathways. Cell Immunol 1999;194:36–46.

[37] Jun CD, Oh CD, Kwak HJ, Pae HO, Yoo JC, Choi BM, Chun JS, Park RK, Chung HT. Overexpression of protein kinase C isoforms protects RAW264.7 macrophages from nitric oxide-induced apoptosis: involvement of c-Jun N-terminal kinase/stress-activated protein kinase, p38 kinase, and CPP-32 protease pathways. J Immunol 1999;162:3395–401.

[38] Hsu SC, Gavrilin MA, Tsai MH, Han J, Lai MZ. p38 mitogen-activated protein kinase is involved in Fas ligand expression. J Biol Chem 1999;274:25769–76.

[39] Huang C, Ma WY, Maxiner A, Sun Y, Dong Z. p38 kinase mediates UV-induced phosphorylation of p53 protein at serine 389. J Biol Chem 1999;274:12229–35.

[40] Nakata M, Uto N, Maruyama I, Yada T. Nitric oxide induces apoptosis via Ca^{2+}-dependent processes in the pancreatic beta-cell line MIN6. Cell Struct Funct 1999;24:451–5.