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Expression of c-Jun in pancreatic islet α-cells of nonobese diabetic(NOD) mice

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Abstract. This is the first report of c-Jun protein expression and mRNA in a pancreatic islet in a nonobese diabetic(NOD) state mice. In this experiment NOD mice with insulin-dependent diabetes mellitus type I at age 16 weeks(n=7) just before death(n=4) were used. The control group consist of prediabetic NOD(8 weeks, n=7) and ICR(8 weeks, n=7 and 16 weeks, n=7) mice. c-Jun positive cells in the pancreatic islet of NOD mice were localized in the same positions as α-glucagon producing cells. Immunoreactivity was negative in the prediabetic NOD(8 weeks) and ICR(8 weeks and 16 weeks) mice. The number of c-Jun positive cells in mice with severe diabetic state just before death were significantly decreased when compared to NOD(16 weeks) mice. Expression of c-Jun in mRNA level was assessed by RT-PCR method. The levels of mRNA in NOD(16 weeks) mice group were elevated in total pancreatic tissues. The present results suggest that the induction of proto-oncogene protein may be of significance in assessing cell specific injury and may play a functional role between pancreatic islet α-cells and β-cells in the diabetic state.

Key words: c-Jun, nonobese diabetic mice(NOD), pancreatic islet α-cell

Introduction

Nonobese diabetic mouse(NOD) represents spontaneous insulin-dependent diabetic mellitus (IDDM) due to the autoimmune destruction of pancreatic β-cells. These mice have been extensively studied as an *in vivo* animal model to define cell and tissue mechanisms of pancreatic β-celll death in the development of human IDDM.[1-4] Death may occur via immune or nonimmune disorders caused by endogenous and exogenous factors which lead to progressive loss of pancreatic β-cells, culminating in severe insulin deficiency. The IDDM mechanism is still unclear. While most studies have focused on the destruction of β-cells, there are no data on the function of non β-cells in relation to β-cell destruction in the diabetic state. The proto-oncogene c-Jun is induced as an immediate response to certain growth factors and phorbol esters.[5-7] The activation of this enzymes family may in part modulate gene expression due to the ability to effect changes in the activity of transcription factors such as AP-1. AP-1 has been identified as the transcriptional product of several c-jun and c-fos proto-oncogene family.

These two factors are highly associate with each other to generate stable heterodimers which have high DNA binding activity. Also, AP-1 mediates transcriptional induction in response to activators of protein kinase C, such as the phorbol esters 12-0-tetradecanoylphorbol 13-acetate(TPA) and phorbol myristate acetate(PMA), through binding to a conserved DNA element(5'TGACTCA-3') known as the TPA-response element.[8-10] The present work examined whether diabetic state is associated with induction of c-Jun expression. Since little is known about the involvement of proto-oncogene in the pancreatic islets of the diabetic state, it was of interest to assess its localization in the pancreatic islets of animals characterizing the diabetic syndrome. This study demonstrates that type 1 diabetic state induces c-Jun mRNA and protein expression.

Materials and Methods

Animals

NOD female mice groups at 16 weeks(n=7), and mice groups of more than 16 weeks (n=4) of age in severe diabetic state before death were used. Control groups consisted of NOD(8 weeks, n=7) and ICR female mice(8 weeks, n=7 and 16 weeks, n=7). The animals were maintained on a 12hr light/dark cycle and fed with autoclaved basal diet and water *ad libitum*. The concentration of urine glucose in NOD mice was determined uropaper(Eiken chemical co., LTD) and were classified as diabetic indicators above 250 mg/dl. Clinical signs of NOD mice(16 weeks, <16 weeks) in the initial diabetic state should wasting(16 weeks) and tremour(<16 weeks)

Immunohistochemistry

Pancreas was rapidly removed after decapitation, cut into 4μm slices, deparaffinized in xylene twice for 5min and rehydrated through graded alcohols. Endogenous peroxidase was blocked by incubating with 0.03% hydrogen peroxide-methanol for 40 min. Sections were washed with PBS containing 0.03 % non-fat milk and 0.01% Tween 20, and then immunostained with rabbit polyclonal c-Jun anti-sera(1:50, Santa Cruz Biotechnology, Inc., CA, USA) overnight at 4℃. Also, we compared the co-localization of glucagon anti-sera(1:600, Dako Co., USA) in c-Jun positive cells through serial sections. The antigen-antibody complex was stained by the avidin-biotin complex(ABC) method using the ABC kit(Vector Co., USA). The color was visualized with 3',3'-diaminobenzidine(DAB; Zymed, San Francisco, CA, USA) as the chromogen.

Oligonucleotides primers

Specific c-jun[11]and glyceraldehyde-3-phosphate dehydrogenase primers[12] were designed from Gene Bank data using a computer program and synthesized by using a DNA synthesizer(Bioneer, LTD., Korea). PCR using specific oligonucleotides primers resulted in DNA products for 443 c-Jun base pairs and 448 GAPDH base pairs.

-Mouse c-Jun (443bp)
sense: 5'-CGG ACC TTA TGG CTA CAG TAA C-3'
anti-sense: 5'-CGT AGA CCG GCG GCT CGC TG-3'

-Mouse GAPDH (448bp)
sense: 5'-ATG GTC TAC ATG TTC CAG TAT GAT TCT-3'
anti-sense: 5'-CAC AGT CTT CTG AGT 7GGC AGT -3'

RNA extraction

Pancreatic total RNA was extracted from pancreas tissues using the Trizol reagent (Gibco BRL) according to the manufacture's protocol. Briefly, the whole pancreas was homogenized in 4ml of Trizol reagent and precipitated with an equal volume of isopropanol and kept at 20℃ for 2hr. The sample was centrifuged at 12,000X g for 15min to obtain the RNA pellet, which

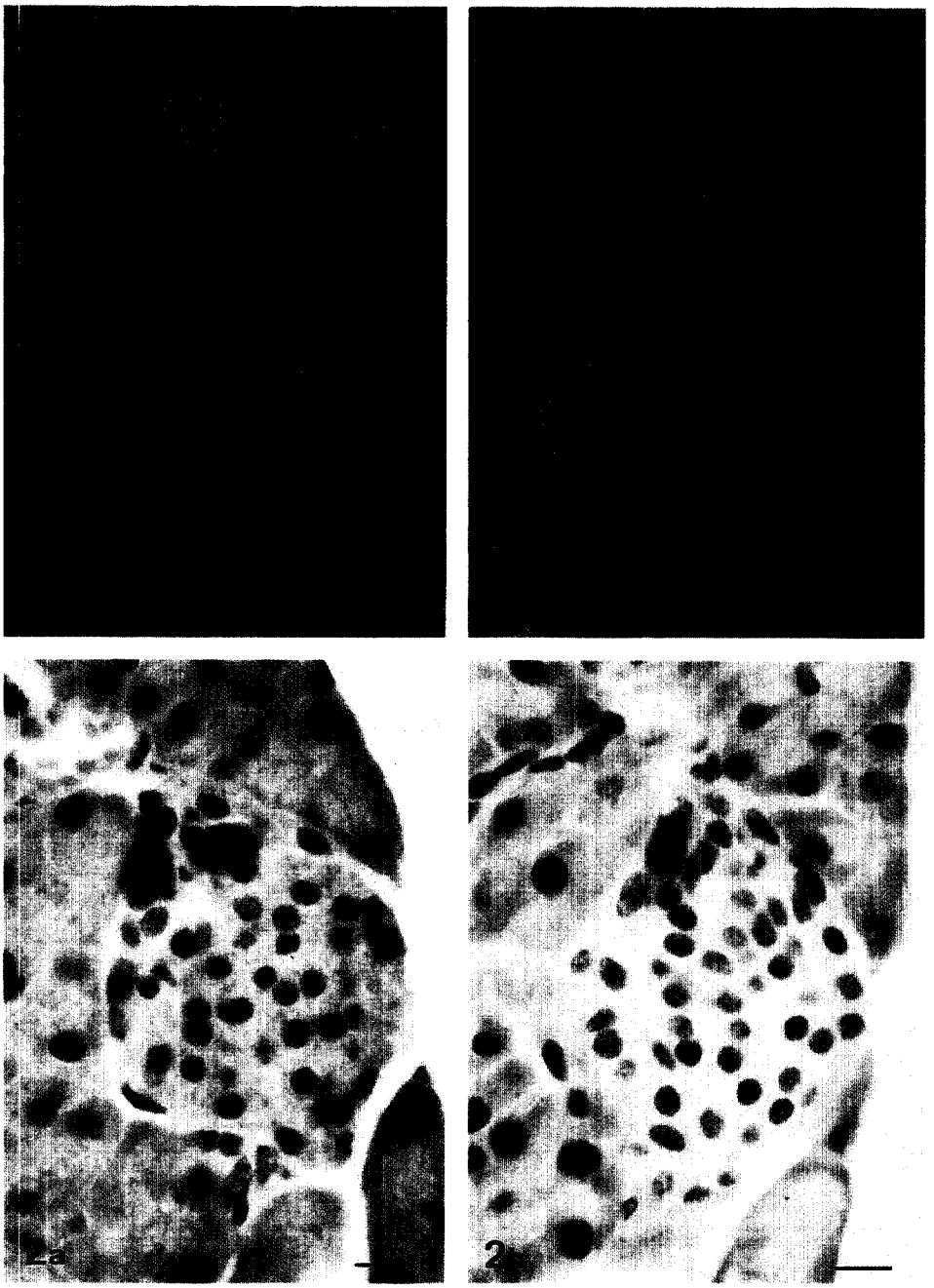

Fig. 1a. Immunohistochemical identification of c-Jun protein in pancreatic islet α-cell of NOD mice at 16 weeks. Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=20㎛.

Fig. 1b. Immunohistochemical identification of glucagon in pancreatic islet α-cell of NOD mice at 16 weeks. Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=20㎛.

Fig. 2a. Immunohistochemical identification of c-Jun in pancreatic islet α-cell of NOD mice until before death. Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=20㎛.

Fig. 2b. Immunohistochemical identification of glucagon in pancreatic islet α-cell of NOD mice until before death. Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=20㎛.

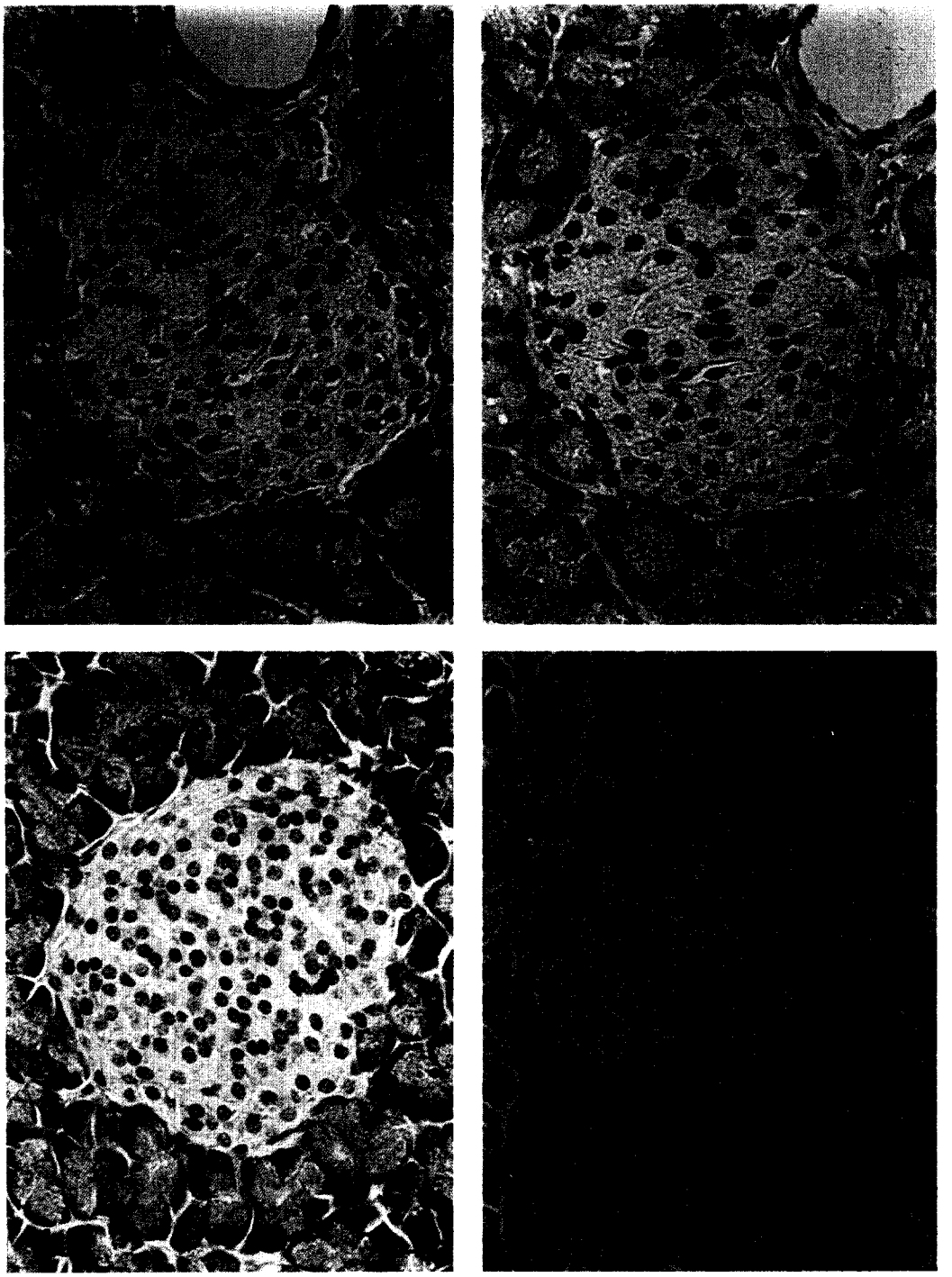

Fig. 3a. No immunereactivity of c-Jun protein in pancreatic islet α-cell of NOD mice at 8 weeks in the prediabetic state. Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=40μm.

Fig. 3b. Immunohistochemical identification of glucagon in pancreatic islet α-cell of NOD mice at 8 weeks. Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=40μm.

Fig. 4a. No immunereactivity of c-Jun protein in pancreatic islet α-cell of normal ICR mice at 16 weeks. Avidi-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=40μm.

Fig. 4b. Immunohistochemical identification of glucagon in pancreatic islet α-cell of ICR mice at 16 weeks. Avidin-biotin-peroxidase method, Mayer's hematoxylin counterstain. Bar=40μm.

was washed with cold 70%(v/v) ethanol 3 times for consistency, dried under vacuum and resuspended with diethylpyrocarbonate-treated H_2O. RNA yield and purity was determined by measuring optical density at 280nm and 260nm.

RT-PCR

Reverse transcription of RNA was carried out in 20$\mu\ell$ of final volume containing 1 mM of each dNTPs, moloney murine leukemia virus reverse transcriptase(1000 units), 1 mM $MgCl_2$, 5 nmol of Oligo d(T), and 10 units of human placental RNAse inhibitors, and 80 ng of pan- creatic total RNA. Amplification reaction was allowed to proceed at 37℃ for 1hr, at 99℃ for 5 min, at 5℃ for 5 min, then quickly chilled at -20℃ for 30 sec, and at 4℃ for 4 min. To the 20$\mu\ell$ of synthesized cDNA, the following components consisted of the following; 1 mM of each dNTP, and 0.2 μM of oligonucleotides primers, 1 units of Taq DNA polymerase(5U/$\mu\ell$), made up in a final concentration of 1X PCR reaction buffer(PCR conditions: 95℃ for 1 min, 56℃ for 1 min, and 72℃ for 1 min.

Results

Specific c-Jun positive cells were confined to pancreatic islet α-cells of diabetic NOD mice(16 weeks and <16 weeks) by immunohistochemistry(Fig. 1a). These cells were positively identified as pancreatic islet α-cells with glucagon anti-sera in serial sections(Fig. 1b). The number of c-Jun and glucagon positive cells decreased in pancreatic islet of NOD mice in severe diabetic state before death(<16 weeks), compared to NOD mice(16 weeks)(Figs. 2a,b). There was no immunoreactivity of c-Jun protein in the prediabetic NOD mice aged 8 weeks(Figs. 3a,b) and ICR mice aged 16 weeks(Figs. 4a,b).

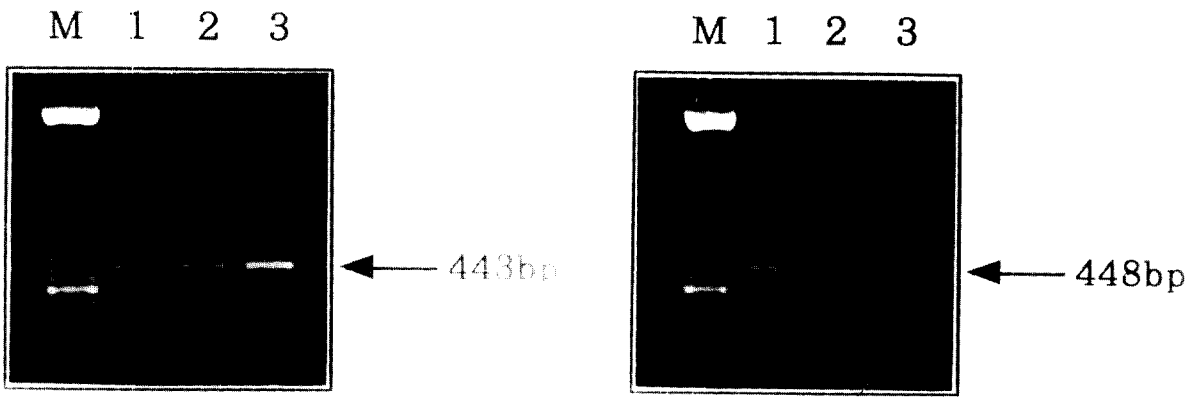

Fig. 5. Reverse transcription-PCR analysis. cDNA was synthesized from the total cellular RNA (40 ng) from pancreas as a template with random hexamer. M: 50 bp size of DNA size markers as a ΦX174DNA/HaeIII (Gibco BRL, N.Y., U.S.A), Lane 1: ICR mice (16 weeks), Lane 2: NOD mice (8 weeks), Lane 3: NOD mice of diabetic state (16 weeks). A specific mouse c-Jun (upper one) and GAPDH (bottom one) were amplified by PCR using 100 pmol of anti-sense and sense. Amplified amounts of specific c-jun mRNA was induced 4-fold in NOD mice while having no changes of GAPDH, indicating specific inducibility.

RT-PCR, which is similar to the immunohistochemistry, provided specific expression of c-Jun in pancreas of all groups and especially overexpressed in the diabetic NOD mice(Fig. 5). The expression of c-Jun mRNA by RT-PCR corre sponded with that of protein levels in pancreatic islets.

Discussion

Several investigators have reported pancreatic β-cell destruction caused by T-cell,[13,14] cytokine and macrophage activities,[15] free-oxygen radical formation resulting in lipid peroxidations,[16] and apoptotic mechanism.[13] Pancreatic α-cells synthesize and secrete glucagon, a hormone involved in regulation of glucose homeostasis. In the diabetic state, it is associated with the β-cell and with factors influencing β-cell destruction. It is known that diabetic syndrome is caused by diverse factors such as cytokines, oxygen radicals, toxic drugs and other xenobiotics.[13 16] In this study, c-Jun proto-oncogene protein was induced in pancreatic islet α-cells during the β-cells destruction, but c-Jun protein was not localized in pancreatic islet β-cells as expected. This induction may probably play pivotal role in influencing β-cell destruction in the diabetic state. The c-Jun proto-oncogene is an immediate response gene which is highly regulated, and its expression is dependent on the state of growth and differentiation as well as external stress factors.[17] Many previous studies demonstrated the involvement of endo-and exogenous factors. Among them, protein kinase C involvement which regulates phosphoinositide(PI)-specific phospholipase C in hepatocytes[20], and the stimulation of phospholipase C associated with DAG production with potential for activation of protein kinase C(PKC). Although the exact biochemical function of most proto-oncogene products is not understood, several of them are known to be involved in various signal transduction such as cell differentiation, promotion and tumor neogenesis.[18,19] Since little is known about the expression of proto-oncogene proteins in the pancreatic islet of normal or diabetic state, our study is of interest in the assessment of their localization in the pancreatic islet of animals with diabetic sym-drome. The overexpression of c-Jun may have an influence on the function of the α-cell as a result of functional impairment of hormone regulation during the β-cell destruction. From these results, it is assumed that a relationship may exist between the high diabetes incidence and the involvement of c-Jun expression in NOD mice. In addition, further studies of the induction of Jun on diabetic state animal should offer new perspectives on the pathogenesis in the pancreatic disease.

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