Activation of NF-κB by Extracellular Matrix Is Involved in Spreading and Glucose-stimulated Insulin Secretion of Pancreatic Beta Cells*

Eva B. Hammar‡§, Jean-Claude Irminger‡, Katharina Rickenbach‡, Géraldine Parnaud‡, Pascale Ribaux‡, Domenico Bosco¶, Dominique G. Rouiller‡, and Philippe A. Halban‡

From the Department of Genetic Medicine and Development, University Medical Center and Cell Isolation and Transplantation Center, University Hospital, 1211 Geneva 4, Switzerland

Laminin-5-rich extracellular matrix derived from 804G cells (804G-ECM) engages β1 integrins to induce spreading, improve glucose-stimulated insulin secretion (GSIS), and increase survival of pancreatic beta cells. The present study examines whether 804G-ECM activates the transcriptional activity of NF-κB and the involvement of NF-κB in those effects of 804G-ECM on pancreatic beta cells. 804G-ECM induces nuclear translocation and the DNA binding activity of the p65 subunit of NF-κB. 804G-ECM-induced nuclear translocation of NF-κB was weak as compared with that induced by interleukin-1β. Transient 804G-ECM-induced DNA binding activity of NF-κB (peak at 2 h) and overexpression of NF-κB target genes IκBa and NF-κB1(p105) (peak at 4 h) were observed. When NF-κB was inhibited by an inhibitor of IκBa phosphorylation (Bay 11-7082) or by a recombinant adenovirus expressing the nonphosphorylatable form of IκBa, 804G-ECM-induced cell spreading and actin cytoskeleton organization were reduced. GSIS from cells on 804G-ECM was inhibited 5-fold, whereas cell survival was not affected. In summary, the results indicate that 804G-ECM induces a transient and moderate NF-κB activity. This study shows for the first time that ECM-induced NF-κB activity is necessary in maintaining GSIS, although it does not affect survival of pancreatic beta cells. The effects of ECM-induced NF-κB activity contrast with the deleterious effects of cytokine-induced NF-κB activity. It is proposed that transient and moderate NF-κB activity is essential for proper function of the pancreatic beta cell.

Engagement of cells by cognate components of the extracellular matrix (ECM) is crucial for various biological processes, including cell adhesion, spreading, proliferation, differentiation, migration, apoptosis, and gene induction. This engagement involves cell adhesion mediated by integrins, a family of heterodimeric molecules composed of an α and a β subunit, with a long extracellular domain binding to the ECM, and a short cytoplasmic domain associating with the actin cytoskeleton and affiliated proteins. ECM is an important component of the pancreatic islet microenvironment. It is a dynamic complex of different molecules that serve as a cellular scaffold regulating both differentiation and survival.

Our group has reported recently that the laminin-5-rich ECM secreted by 804G cells (804G-ECM) induced increased spreading and improved insulin secretion in response to glucose in purified pancreatic beta cells. It was subsequently shown that this ECM has a beneficial effect on cell survival and that it activates intracellular signaling pathways involving the signaling proteins focal adhesion kinase, Akt/PKB, and ERK. It has been shown that its effects on pancreatic beta cells (spreading, glucose-stimulated insulin secretion, and survival) are mediated by the engagement of β1 integrins to laminin-5.

Furthermore, it was found that plating pancreatic beta cells on 804G-ECM causes overexpression of the inhibitor of NF-κB (IκBa) (4), which is a well established NF-κB target gene. This finding led us to hypothesize that 804G-ECM might activate the NF-κB signaling pathway, leading to the expression of a subset of genes, which might be involved in the effects of 804G-ECM on the pancreatic beta cell. Engagement of integrins to different ECM molecules (vitronectin, fibronectin, and laminin) has been shown to activate NF-κB and to promote an NF-κB-dependent program of gene expression.

This paper is available online at http://www.jbc.org

* This work was supported by Grant 3200-06177.00 from the Swiss National Science Foundation, Grant 4-2002-461 from the Juvenile Diabetes Research Foundation, and an unrestricted educational grant from Novo Nordisk A/S, Copenhagen. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Genetic Medicine and Development, University Medical Center, Rue Michel Sertel 1, 1211 Geneva 4, Switzerland. Tel.: 41-22-379-5537; Fax: 41-22-379-5528; E-mail: eva.hammar@medecine.unige.ch.

§ The abbreviations used are: ECM, extracellular matrix; GSIS, glucose-stimulated insulin secretion; NF-κB, nuclear factor-κB; IκBa, IκBα, inhibitor of NF-κB; IκBα; phospho-ERK, nonphosphorylatable IκBα mutant; ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; pLL, poly-(L-lysine); PKB, protein kinase B; ELISA, enzyme-linked immunosorbent assay; MAP, mitogen-activated protein; PBS, phosphate-buffered saline; GFP, green fluorescent protein; TUNEL, terminal dUTP nick-end labeling; IL, interleukin.

G. Parnaud, unpublished results.
be down-regulated by multiple mechanisms, including the well characterized feedback pathway involving newly synthesized IkB proteins (12, 15, 16).

In the pancreatic beta cell, NF-κB is considered as an important transcription factor mediating IL-1β-induced signal transduction and regulating groups of genes contributing to death and dysfunction (17). Indeed, inhibition of NF-κB has been shown to protect against the deleterious effects of IL-1β in purified mouse islets (18), rat beta cells (19), and human islets (20, 21). Given that NF-κB has thus been considered previously to be pro-apoptotic in the beta cell, the possibility that activation of NF-κB signaling by 804G-ECM may be beneficial for beta cell function and/or survival was quite intriguing. The aims of this study were to investigate whether 804G-ECM activates the NF-κB transcriptional activity in primary pancreatic beta cells, to study the kinetics of this activity, and to determine the involvement of this pathway on the effects of 804G-ECM on the pancreatic beta cell. Here we show that 804G-ECM induces transient nuclear translocation of NF-κB and its transcriptional activity in pancreatic beta cells, which is followed by overexpression of IkBα and NF-κB mRNAs. Blocking the MAP kinase ERK pathway with PD98059 inhibited the DNA binding activity of NF-κB and overexpression of IkBα, whereas the PI3K inhibitor LY294002 did not. We report, for the first time, that long term blockade of NF-κB activity disrupts cytoskeleton remodeling, inhibits spreading induced by 804G-ECM, and reduces glucose-stimulated insulin secretion. Furthermore, we show that induction of NF-κB activity by 804G-ECM is not involved in its control of pancreatic beta cell survival.

MATERIALS AND METHODS

Reagents and Antibodies—Bay 11-7082 (an inhibitor of IkBα phosphorylation) was purchased from BioMol Research Laboratories (Hamburg, Germany). PD98059 (a specific MEK1 inhibitor) and LY294002 (a phosphatidylinositol 3-kinase (PI3K) inhibitor) were purchased from Calbiochem, and both were used at final concentration of 50 μM as described previously (4). The polyclonal antibodies against the p65 subunit of NF-κB (C-20) and against the inhibitors of NF-κB (IkBα, C-21; IkBβ, C-20) were from Santa Cruz Biotechnology (Santa Cruz, CA). A monoclonal anti-actin antibody according to the manufacturer’s instructions. Alexa Fluor® 546 phalloidin was purchased from Molecular Probes. Alexa Fluor® 546 phalloidin (5 units/ml), subsequently rinsed, and mounted under a gelatinous gel was electrophoblotmed into nitrocellulose membranes (Schleicher & Schuell) for immunoblotting with the appropriate antibody. An ECL protein detection kit (Amersham Biosciences) and a Kodak image station were used for visualization of the bands.

Quantitative Real-Time PCR—RNA was isolated using RNeasy mini kit (Qiagen). cDNA was synthesized with Superscript II (Invitrogen), using 1 μg of total RNA in a 20-μl reaction volume. The double-stranded DNA-specific dye SYBR Green I (Eurogentech, Belgium) and fluorescent (Bio-Rad) were incorporated into the PCR buffer (qPCR core kit, Eurogentech) to allow for quantitative detection of the PCR product. The results were analyzed using the iCycler iQ System (Bio-Rad). The hammerhead gene L3 was used as an internal control. The primers used were as follows: NF-κB forward 5′ GTC TAG CAA TCA CGG CTG CA 3′, NF-κB reverse 5′ CTC AGG CCA CCA TAC CCC AA A 3′; IkBα forward 5′ TGC TGA GGC ACT TCT GCC 3′, IkBα reverse 5′ TCC TCG AAA GTC TCG GAG GTC 3′.

Inhibition of NF-κB Activity—Two approaches were used to inhibit NF-κB activity as follows: use of recombinant adenoviruses expressing CαB, transfection with small interfering IκBα (IκBomp), and pharmacological inhibition of IkBα phosphorylation with Bay 11-7082 (24). The recombinant adenoviruses were a kind gift from Dr. Wrede, University of Regensburg, Germany (19, 25). The adenoviruses were titrated using Adeno-X™ Rapid Titer kit (Clontech) according to the manufacturer’s instructions. Cells (104 cells/ml) were infected in suspension of 200 μl at 37 °C with 150 infectious units/ml, washed twice with culture medium, and left in suspension for 24 h before plating them on pLL or on 804G-ECM-coated dishes. To investigate the effect of the inhibition of NF-κB activity using Bay 11-7082, cells were pre-treated for 1 h with 2.5 and 5 μM Bay 11-7082 before plating them on pLL or on 804G-ECM-coated dishes. MeSO was added to the controls at the same final concentration as used for the inhibitors. Cells were then cultured in the continued presence of the inhibitor. The analyses of the effects of IκBomp-expressing virus and of Bay 11-7082 on NF-κB nuclear translocation, cell spreading, cell function (insulin secretion), and cellular death were performed in parallel.

Measurement of Spreading of Cells—After 24 h of culture and treatment of cells as described above, cells were fixed with 4% paraformaldehyde (20 min, room temperature) and stained with Evans Blue. Phase-contrast views of different fields were photographed, and spreading of cells was quantified using ScionImage™ software (Frederick, MD).

F-actin Cytoskeleton Visualization—After 24 h of culture and treatment of cells as described above, cells were fixed with 4% paraformaldehyde (20 min, room temperature) and permeabilized with 0.5% Triton X-100 (4 min at room temperature). After blocking with PBS + 0.5% BSA and 0.4% crystallinealbumin for 30 min, Alexa Fluor® 546 phalloidin (5 units/ml), subsequently rinsed, and mounted under glass coverslips. The preparations were observed with a confocal microscope.

Analysis of Cell Death by TUNEL—Attached cells were washed with ice-cold PBS supplemented with 1 mM sodium vanadate and protease inhibitors and lysed in sample buffer 1× (62 mM Tris-Cl, pH 6.8, 2% SDS, 5% glycerol, 1% 2-mercaptoethanol), protein concentrations were determined with the Amido Black method (23), and equal amounts of total protein were loaded for SDS-PAGE. All samples, after separation on an SDS-polyacrylamide gel, were electrophoblotmed onto nitrocellulose membranes (Schleicher & Schuell) for immunoblotting with the appropriate antibody. An ECL protein detection kit (Amersham Biosciences) and a Kodak image station were used for visualization of the bands.
Extracellular Matrix, NF-κB Activity, and Insulin Secretion

3-ΟH strand breaks resulting from DNA degradation) was performed with the In Situ Cell Death Detection kit, according to the manufacturer’s instructions (Roche Applied Science). The preparations were then rinsed with PBS and incubated (15 min, room temperature) with 1 μg/ml Hoechst 33342 to allow detection of nuclei and to facilitate the analysis. The quantification of dead cells was performed by using an Axiocam fluorescence microscope.

**Insulin Secretion Assay and Insulin Content**—Cells were washed three times with a modified Krebs-Ringer bicarbonate HEPES buffer (KRBH: 125 mM NaCl, 4.74 mM KCl, 1 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 5 mM NaHCO₃, 25 mM HEPES, pH 7.4, 0.1% bovine serum albumin) supplemented with 2.8 mM glucose and preincubated with this same buffer for 1 h at 37 °C. Cells were then incubated for 1 h at 37 °C with KRBH containing 2.8 mM glucose (basal secretion), followed by 1 h at 37 °C with KRBH containing 16.7 mM glucose (stimulated secretion). The incubation buffer was recovered, and the cells were extracted with acid/ethanol. The buffer was centrifuged to remove any detached cells and debris. Aliquots were stored at −20 °C for subsequent insulin measurement performed by radioimmunassay using the charcoal separation technique described previously (26). GSIS is expressed as secreted insulin upon glucose challenge (16.7 mM glucose, for 1 h), as a percentage of insulin content. Insulin content is expressed as the quantity of insulin extracted at the end of the stimulation test. The total measurement performed by radioimmunoassay using the charcoal separation technique described previously (26).

**Presentation of Data and Statistical Analysis**—Unless stated otherwise, data are presented as mean ± S.E. for “n” independent experiments, and levels of significance for differences between groups were assessed by Student’s t test for unpaired groups.

**RESULTS**

**804G-ECM Induces NF-κB Nuclear Translocation**—This study was performed with laminin-5-rich extracellular matrix derived from 804G rat bladder carcinoma cells (804G-ECM). 804G-ECM induces attachment and spreading of rat primary beta cells, because of engagement of β1 integrins by laminin-5 contained in this ECM (3, 4). 804G-ECM is thus used as a validated model to study signaling pathways activated by this engagement.

In the canonical NF-κB pathway, one of the first steps involved in NF-κB-dependent gene regulation is the phosphorylation and subsequent degradation of the inhibitor of NF-κB (IκBα), leading to the nuclear translocation of the p65 subunit of NF-κB (RelA). To investigate a possible involvement of 804G-ECM in NF-κB transcriptional activity, the cellular localization of NF-κB after short term exposure (1 h) to 804G-ECM compared with control (pLL-coated dishes) was assessed by immunofluorescence for the p65 subunit of NF-κB (Fig. 1A). The number of cells with nuclear NF-κB localization was quantified (Fig. 1B). 804G-ECM induced a significant increase in the number of cells with nuclear NF-κB (19%) compared with control (less than 5%; Fig. 1B). The cytoplasmic staining for NF-κB was still apparent in all cells on 804G-ECM, suggesting that only a fraction of the total pool of cytoplasmic NF-κB is translocated into the nucleus in response to 804G-ECM (Fig. 1A). By contrast, treatment of cells for 20 min with 2 ng/ml IL-1β induced the nuclear translocation of p65 in all treated cells, and this was complete as there was no more cytoplasmic staining for NF-κB (not shown).

**804G-ECM Induces NF-κB Transcriptional Activity**—To assess whether DNA binding activity of NF-κB is induced by 804G-ECM, the amount of p65 complexes binding to oligonucleotides containing an NF-κB consensus binding site was quantified by ELISA. The DNA binding activity of NF-κB in cells cultured on 804G-ECM-coated dishes as compared with control was increased after 1 h of culture, and this increase was significant after 2 h of culture (Fig. 2). This activity was decreased after 4 h of culture suggesting that 804G-ECM-induced NF-κB DNA binding activity is transient. It is well established that activated NF-κB induces expression of IκBα. Newly synthesized IκBα binds to NF-κB, providing a negative feedback loop for the NF-κB signaling pathway (12). Activated NF-κB is also able to induce the expression of IκBα1 (p105) in several systems (11, 27). To investigate whether 804G-ECM is able to induce NF-κB transcriptional activity, IκBα and NF-κB1 mRNA levels were quantified by real time PCR in cells cultured on pLL- or on 804G-ECM-coated dishes. IκBα mRNA levels were increased after 2 h on 804G-ECM, and both IκBα and NF-κB1 mRNA levels were significantly increased by 804G-ECM after 4 and 8 h of culture to return to control (pLL) levels at 24 h (Fig. 3, A and B). The kinetics of 804G-ECM-induced IκBα and NF-κB1 gene expression thus correlate with those of NF-κB DNA binding activity (Fig. 2). In summary, these data indicate that 804G-ECM induces NF-κB transcriptional activity transiently, most probably as a consequence of feedback inhibition resulting from increased expression of IκBα.

**Regulation of IκBα and IκBβ Protein Levels by 804G-ECM**—To investigate whether 804G-ECM induces IκBα and/or IκBβ degradation, Western blots were performed with protein extracts from cells cultured for 1 h on pLL or on 804G-ECM-coated dishes. Quantification of the resulting bands normalized to actin showed that there was no significant decrease of IκBα and IκBβ protein levels on 804G-ECM as compared with pLL after 1 h of culture (Fig. 4). IκBα and IκBβ protein levels were also analyzed after 24 h of culture on pLL- or on 804G-ECM-coated dishes. Levels of IκBα...
protein were significantly increased on 804G-ECM after 24 h of culture as compared with pLL and as compared with 804G-ECM after 1 h of culture (Fig. 4). By contrast, IxBβ protein levels were not altered with time or by the substrate used. Although NF-κB induces expression of IxBα, it does not control expression of IxBβ (28). Therefore, these results suggest that the 804G-ECM-induced IxBα overexpression occurs specifically through NF-κB.

Inhibition of NF-κB Activity; Effects on Spreading and Actin Cytoskeleton Remodeling—To investigate the function of the 804G-ECM-induced NF-κB activity, Bay 11-7082, an inhibitor of IxBα phosphorylation and thus of NF-κB nuclear translocation (24), was used. Bay 11-7082 induced a dose-dependent decrease of 804G-ECM-induced NF-κB nuclear translocation, and a final concentration of 5 μM of Bay 11-7082 was necessary to induce a complete and significant decrease compared with control condition (Fig. 5A). Similar results were observed when the effect of this inhibitor on 804G-ECM-induced NF-κB DNA binding activity was assessed by ELISA (not shown). Furthermore, Bay 11-7082 inhibited overexpression of IxBα mRNA induced by 804G-ECM, thus confirming that it inhibits the transcriptional activity of NF-κB (Fig. 8B). We have shown previously (3) that 804G-ECM induces spreading of the pancreatic beta cell, and this effect is glucose-dependent. NF-κB has been shown to be involved in cell motility through its effect on gene expression (reviewed in Ref. 29), leading us to hypoth-

![Fig. 3. NF-κB and IxBα mRNAs levels are regulated by 804G-ECM.](Image 356x366 to 524x737)
on 804G-ECM in the presence of the inhibitor were less spread, close inspection of the confocal images (Fig. 5C) suggests a better developed and more consequent cortical actin ring in the treated cells.

Recombinant adenovirus expressing nondegradable IκBα (IκBnp) has been shown to be an effective inhibitor of NF-κB nuclear translocation and transcriptional activity (19, 20). 804G-ECM-induced nuclear translocation of NF-κB was decreased in cells transduced with the recombinant IκBnp virus as compared with cells transduced with the control (GFP) adenovirus (data not shown). The effect of IκBnp on cell spreading was analyzed after 24 h of culture on pLL or on 804G-ECM. Spreading of cells infected with IκBnp was reduced compared with cells infected with the control virus (Fig. 6), confirming that 804G-ECM-induced NF-κB activity is involved in the spreading of pancreatic beta cells induced by 804G-ECM.

Inhibition of NF-κB Activity, Effects on Insulin Secretion—The importance of cell-matrix interactions for optimal GSIS (insulin secreted at 16.7 mM glucose) by pancreatic beta cells in culture has been demonstrated by us (3) and by other groups (31–36). However, the mechanism involved has yet to be clarified. It has been suggested that there is a correlation between increased spreading of cells with their ability to respond optimally to glucose (3). As NF-κB activity seems to be involved in spreading of cells and organization of the actin cytoskeleton induced by 804G-ECM (Figs. 5 and 6), we hypothesized that it might be involved in the beneficial effects of 804G-ECM on GSIS. As shown in Fig. 7A and in Table I, GSIS of cells cultured for 24 h on 804G-ECM was significantly increased compared with cells cultured with pLL. Bay 11-7082 induced a dose-dependent decrease of GSIS of cells cultured on 804G-ECM, and a final concentration of 5 μM of this inhibitor induced a significant decrease of GSIS in cells on both pLL and 804G-ECM (Fig. 7A and Table I). Furthermore, the fold stimulation of insulin secretion (amount of insulin secreted at 16.7 mM glucose compared with that secreted at 2.8 mM glucose) was significantly decreased in treated cells, both on pLL and on 804G-ECM (Table I). In addition, Bay 11-7082 induced a significant decrease in “absolute” stimulated insulin secretion (i.e. amount of secreted insulin at 16.7 mM glucose, per dish and hour) as well as an increase in total insulin content of cells plated on 804G-ECM, as compared with the control condition (Table I). Cellular protein content was similar in all conditions (Table I). Bay 11-7082 thus inhibits insulin secretion. In summary, these results indicate that 804G-ECM-induced NF-κB activity is involved in spreading, actin cytoskeleton organization, and GSIS of pancreatic beta cells.

Inhibition of ECM-induced NF-κB Activity Does Not Affect Cell Survival—804G-ECM protects pancreatic beta cells against apoptosis (4), so we hypothesized that NF-κB might mediate its pro-survival effect. TUNEL assays were performed on cells cultured for 24 h on pLL or on 804G-ECM-coated dishes, in the absence or in the presence of Bay 11-7082. Treatment of cells with 5 μM Bay 11-7082 did not affect cell survival either on pLL or on 804G-ECM (Fig. 7B). Similar results were obtained using adenoviruses expressing nonphosphorylatable IκBα (data not shown). Therefore, it seems that 804G-ECM-induced activity of NF-κB is not involved in the control of survival and/or apoptosis of pancreatic beta cells.

Involvement of the MAP Kinase ERK Pathway in 804G-ECM-induced NF-κB Activity—We have shown previously that 804G-ECM induces phosphorylation of the MAP kinases ERK1 and ERK2, as well as of Akt/PKB, and that both pathways seemed to be involved in the anti-apoptotic effect of 804G-ECM (4). The signaling pathways involving ERK and Akt/PKB have both been reported to be able to activate NF-κB (5, 10, 29, 37). To get a preliminary insight into how 804G-ECM activates NF-κB transcriptional activity, the effects of PD98059 (inhibitor of MEK1 and thereby of the MAP kinase ERK pathway) and of LY294002 (inhibitor of PI3K and thereby of Akt/PKB signaling) on the DNA binding activity of NF-κB and on the overexpression of IκBα induced by 804G-ECM were analyzed. As shown in Fig. 8A, PD98059 inhibited the NF-κB DNA binding activity by 40% (p < 0.0005). We have shown previously that PD98059 inhibits the overexpression of IκBα induced by 804G-ECM (4). Taken together, these results suggest that the MAP kinase ERK pathway might be involved in the activation of NF-κB induced by 804G-ECM. By contrast, the inhibitor...
Involvement in the regulation of the transcriptional activity of NF-κB. The overexpression of IκBα was sustained for at least 48 h (4). This prolonged by 804G-ECM. Overexpression of IκBα would be that only a minor fraction of NF-κB protein is translocated to the nucleus. The fact that the cytoplasmic fraction of the total IκBα is most interesting, because increased NF-κB nuclear translocation and removal NF-κB from its nuclear binding sites after 1 h of culture on 804G-ECM was not significant as compared with control or with LY294002 (50 μM) had no effect either on the DNA binding activity of NF-κB (Fig. 8 A) or on the expression of IκBα (Fig. 8B), suggesting that the PI3K-Akt/PKB pathway is not involved in the regulation of the transcriptional activity of NF-κB by 804G-ECM.

**DISCUSSION**

In this report we show that 804G-ECM induces nuclear translocation of NF-κB, that it increases binding of NF-κB to DNA, and that it induces upregulation of the well established NF-κB target genes NF-κB1 (p105) and IκBα. Collectively, these data indicate that 804G-ECM induces NF-κB transcriptional activity in primary pancreatic β cells. Others have reported that various ECMs are able to activate NF-κB in different cell types; however, most studies have been performed in cell lines (transformed or not), and to our knowledge there has been no such study in primary, nondividing cells.

In the canonical pathway leading to NF-κB activation, others have observed a substantial degradation of IκBα prior to NF-κB nuclear localization. In our system, the degradation of IκBα after 1 h of culture on 804G-ECM was not significant as compared with control (pLL), suggesting that only a small fraction of the total IκBα pool is degraded. The consequence would be that only a minor fraction of NF-κB cytosolic dimers is translocated to the nucleus. The fact that the cytoplasmic staining for NF-κB was still apparent in all cells exposed for 1 h to 804G-ECM sustains the latter hypothesis.

After 24 h of culture, IκBα protein levels were significantly increased on 804G-ECM as compared with pLL, and this increase was sustained for at least 48 h (4). This prolonged overexpression of IκBα is most interesting, because increased IκBα expression will both prevent additional NF-κB nuclear translocation and remove NF-κB from its nuclear binding sites (12) and could thus be responsible for the observed transience of 804G-ECM-induced NF-κB activity.

Several different signaling proteins have been reported to mediate signaling from ECM to NF-κB, including MAP kinase ERK (37), PI3K (5, 10), and Rho GTPase Rac (5, 10, 38). We have reported previously (4) that the MAP kinase-ERK and PI3K-Akt/PKB pathways are activated by 804G-ECM, suggesting that these latter pathways might be involved in 804G-ECM-induced NF-κB activity. Based on the use of selective inhibitors, we now show that the MAP kinase ERK pathway, but not the PI3K-Akt/PKB pathway, mediates the increased DNA binding activity of NF-κB as well as overexpression of IκBα induced by 804G-ECM.

As reported previously by our group, plating beta cells on 804G-ECM induces their attachment, cytoskeleton remodeling, and cell spreading. Here we report that long term blockage of NF-κB activity by pharmacological means (Bay 11-7082) or by adenoviral overexpression of nonphosphorylatable IκBα impairs 804G-ECM-induced spreading of the cells. This inhibitory effect might depend on expression of genes induced by NF-κB. Indeed, it has been reported by other groups that NF-κB can mediate overexpression of proteins involved in spreading and/or migration of cells (29).

Furthermore, we have observed that blocking NF-κB activity with Bay 11-7082 impairs GSIS, but it does not affect cell survival. While this work was in preparation, Norlin et al. (39) reported the effects of attenuating basal NF-κB activity on GSIS and on cell survival, using transgenic mice expressing nonphosphorylatable IκBα. Most interestingly, although their

**TABLE 1**

| Effect of inhibition of 804G-ECM-induced NF-κB activity on secretion and cell content of insulin |
|------------------------------------------------|
| Cells were pretreated or not with 5 μM Bay 11-7082, plated on pLL-, or 804G-ECM-coated dishes, and cultured for 24 h in the continued presence of this inhibitor. Fold increase indicates insulin secretion at 16.7 mM glucose/2.8 mM glucose. |
| pLL | Bay 11-7082 | 804G-ECM |
|-----------------|------------------|------------------|
| Insulin secretion (16.7 mM Glc) (% content/h) | 4.38 ± 0.06 | 1.36 ± 0.25 | 9.23 ± 1.34 |
| Insulin secretion (2.8 mM Glc) (% content/h) | 0.21 ± 0.04 | 0.21 ± 0.01 | 4.07 ± 0.04 |
| Fold increase | 10.7 ± 1.7 | 6.1 ± 1.15^* | 19.7 ± 4.6 |
| Insulin secretion (16.7 mM Glc) (ng/dish × h) | 14.96 ± 1.34 | 5.93 ± 1.01 | 13.17 ± 2.58 |
| Insulin secretion (2.8 mM Glc) (ng/dish × h) | 1.34 ± 0.2 | 0.97 ± 0.13 | 0.67 ± 0.08 |
| Insulin content (ng/dish) | 325.8 ± 31 | 459.2 ± 51.9 | 141.5 ± 7.4 |
| Cellular protein content (μg/dish) | 14.96 ± 4.25 | 4.42 ± 0.4 | 276.5 ± 29.7 |

^* p < 0.01 versus pLL control.
^b p < 0.002 versus 804G-ECM control.
^c p < 0.005 versus control condition on respective substrate.
^d p < 0.02 versus control condition on respective substrate.
^e p < 0.02 versus pLL control.
^p < 0.02 versus pLL Bay 11-7082 (n = 5–6 from three independent experiments). The cellular protein contents were measured in a separate series of experiments (n = 3 from two independent experiments).

![Graph A](image)

**FIG. 8.** A. Effects of inhibitors of the MAP kinase ERK and the PI3K-Akt/PKB pathways (PD98059 and LY294002) on NF-κB DNA binding activity. Cells were pretreated or not with PD98059 (50 μM) or with LY294002 (50 μM) for 15–30 min before plating them on pLL- or on 804G-ECM-coated dishes. NF-κB DNA binding activity (normalized to control) was measured by ELISA (absorbance, 450–650 nm) after 2 h of exposure to 804G-ECM. *, p < 0.0005 (n = 4). B. Effects of NF-κB inhibitor Bay 11-7082 and LY294002 on 804G-ECM-induced overexpression of IκBα mRNA. Cells were pretreated or not with Bay 11-7082 (5 μM) or with LY294002 (50 μM) for 15–30 min before plating them on pLL- or on 804G-ECM-coated dishes. mRNA was extracted after 4 h of culture, and IκBα mRNA levels were measured by reverse transcription-PCR. The results (means ± S.D. of two independent experiments) are shown as IκBα mRNA levels relative to the internal control L3, normalized to control condition (pLL, control).
approach was different from ours, the observed phenotypes because of NF-κB inhibition were similar in both models; GSIS was impaired, whereas beta cell survival was not affected.

We have shown previously that the MAP kinase ERK pathway could be involved in the pro-survival effect of the 804G-ECM but that it does not affect spreading of beta cells (4). As discussed above, this pathway seems to be involved in 804G-ECM induction of NF-κB activity as well. It is intriguing in this context that inhibition of NF-κB did not affect beta cell survival but that it did inhibit spreading of cells. ERK is known to affect the activities of a multitude of signaling pathways and transcription factors (40), regulating migration and survival of cells. Therefore, we propose that ERK effectors other than NF-κB may mediate its pro-survival effect. Spreading and/or migration of cells is an extremely complex process, involving the interplay of several signaling pathways (41). Further studies are therefore mandatory to better dissect the inter-dependence of the MAP kinase ERK and NF-κB pathways as well as other candidate signaling proteins (such as the Rho GTPases) involved in the effects of the 804G-ECM on the pancreatic beta cell.

What is the connection between NF-κB and GSIS? Different mechanisms may be involved, and in our opinion, they might well complete each other. One possible connection between NF-κB and GSIS is the actin cytoskeleton, and another one is regulation of gene expression. The actin filaments are organized in two ways in secretory cells as follows: a cortical F-actin web (rim or ring) underneath the plasma membrane and actin filament fibers distributed throughout the cytosol (42, 43). Many studies, including in pancreatic islets, have reported that the actin web limits the access of the secretory granules to the cell boundary; disruption or remodeling of the web is believed to be a prerequisite for exocytosis (42, 44–46). On the other hand, the function of actin filaments in the cell interior is less well understood. However, dynamic association of insulin-containing granules with actin cytoskeleton is thought to be involved in insulin exocytosis. As shown in this work, 804G-ECM induces remodeling of the actin cytoskeleton, leading to the appearance of actin filament fibers in the cell interior. Upon treatment with Bay 11-7082, these actin fibers were no more apparent, and this may underlie impaired insulin secretion. The cortical actin web appeared more prominent in the treated cells, and this may further impair insulin secretion. Therefore, we suggest that NF-κB might be involved in GSIS through the remodeling of the actin cytoskeleton. Depolarization-induced Ca\(^{2+}\) is a required step for GSIS to occur. Most interestingly, it has been reported that depolarization/Ca\(^{2+}\) influx can activate NF-κB transcriptional activity and that the ERK inhibitor PD98059 blocked this activation of NF-κB (47). However, the consequences of such depolarization-induced NF-κB activity are still unknown (47).

It has been shown that expression of genes implicated in glucose uptake, oxidative metabolism, and Ca\(^{2+}\)-triggered exocytosis is perturbed in transgenic mice expressing non-phosphorylatable IκBα (39). Furthermore, long term treatment of neuronal cells with tumor necrosis factor-α enhances depolarization-induced increases of Ca\(^{2+}\), and it has been suggested that these effects are induced via altered gene expression mediated by NF-κB (48). Therefore, we propose that blockage of 804G-ECM-induced NF-κB activity might repress expression of genes necessary for well regulated GSIS.

It is now well acknowledged that NF-κB plays a major role in cytokine-induced beta cell dysfunction and apoptosis (17). However, this study shows a completely different aspect of NF-κB function in beta cells. A critical question arises: how does a given stimulus lead to specific biological end points through regulation of NF-κB activity? The functional consequences of NF-κB activity seem to depend on several different factors, such as the kinetics, extent, and context of activation of NF-κB. 804G-ECM-induced nuclear translocation of NF-κB appeared to be moderate when compared with that occurring after cytokine treatment. Furthermore, we show that 804G-ECM-induced binding of NF-κB to DNA declines after 2 h of exposure to 804G-ECM and that 804G-ECM-induced overexpression of IκBα and NF-κB1 (p105) mRNAs begins to decay after 8 h of culture, indicating that 804G-ECM-induced NF-κB activity is transient. This contrasts with the sustained activity of NF-κB induced by cytokines. These differences in kinetics and extent of NF-κB activity may explain the different functional outcomes mediated by 804G-ECM- and cytokine-induced NF-κB activation. It has been reported that the first phase of IL-1β-induced NF-κB activity leads to the beneficial increase of beta cell defense/repair protein expression. By contrast, the second phase of NF-κB activity induced by IL-1β is harmful, because it leads to a sustained decrease of specific beta cell proteins like insulin, GLUT-2, and PDX-1 with a concomitant increase of other “aspecific” proteins and inducible nitric-oxide synthase transcription (49). In summary, these data combined with our own data support the concept that transient and/or low NF-κB activity is beneficial, whereas sustained and/or strong NF-κB activity is deleterious for the pancreatic beta cell.

Recently it has emerged that the transactivation potential of NF-κB is modulated by post-translational modifications, including phosphorylation and acetylation of NF-κB subunits as well as of histones surrounding NF-κB target genes (reviewed in Refs. 50 and 51). Site-specific phosphorylation of the p65 subunit of NF-κB can occur by a large variety of kinases in response to different stimuli (51) and may target NF-κB to a particular subset of genes (52). We hypothesize that IL-1β and 804G-ECM might induce phosphorylation of p65 on distinct sites, leading to different biological responses. Experiments aimed at exploring this hypothesis are underway in our laboratory. The next challenge would be to inhibit selectively the NF-κB activity leading to its deleterious effects without interfering with its beneficial outcomes.

The procedure leading to pancreatic beta cell isolation prior to pancreatic islet transplantation involves disruption of cell-cell and cell-ECM contacts (33, 53). The isolated pancreatic beta cells lose glucose responsiveness and eventually die when maintained in culture for a long period of time. By contrast, when layered on appropriate ECM, pancreatic islet cell function and survival can be maintained (3, 4, 31–33, 35, 36, 54). Here we show that ECM induces transient and moderate activity of the transcription factor NF-κB (p65) and that ECM-induced NF-κB activity is involved in spreading, cytoskeleton organization, and improved function (GSIS) of primary pancreatic beta cells. We propose that transient and moderate NF-κB activity is essential for well regulated glucose-stimulated insulin secretion in the pancreatic beta cell.

Acknowledgments—We thank Nadja Perriraz-Mayer and Caroline Raveraud for expert technical assistance. We also thank Barbara Yermen for critical reading of the manuscript.

REFERENCES
1. Miranti, C. K., and Brugge, J. S. (2002) Nat. Cell Biol. 4, E83–E90
2. Juliano, R. L. (2002) Annu. Rev. Pharmacol. Toxicol. 42, 283–323
3. Bosco, D., Meda, P., Halban, P. A., and Rouiller, D. G. (2000) Diabetes 49, 233–243
4. Hammari, E., Parnaud, G., Bosco, D., Perriraz, N., Maeder, K., Donath, M., Rouiller, D. G., and Halban, P. A. (2004) Diabetes 53, 2034–2041
5. Reyes-Reyes, M., Mora, N., Zentella, A., and Rosales, C. (2001) J. Cell Sci. 114, 1579–1589
6. Rosales, C., and Juliano, R. (1996) Cancer Res. 56, 2302–2305

³ T. Mandrup-Poulsen, personal communication.
Extracellular Matrix, NF-κB Activity, and Insulin Secretion

7. Lin, T. H., Rosales, C., Mondal, K., Bolen, J. B., Haskell, S., and Juliano, R. L. (1995) J. Biol. Chem. 270, 16189–16197
8. Scatena, M., Almeida, M., Chaisson, M. L., Fausto, N., Nicosia, R. F., and Giachelli, C. M. (1998) J. Cell Biol. 141, 1083–1093
9. Gwaryntrum, Z. E., Oetberg, C. O., Turk, G. L., Richardson, C. A., and Bomshy, K. (1994) J. Biol. Chem. 269, 30765–30768
10. Klein, S., de Fougerolles, A. R., Blakie, P., Khan, L., Pepe, A., Green, C. D., Kotelniansky, V., and Giancotti, F. G. (2002) Mol. Cell. Biol. 22, 5912–5922
11. de Fougerolles, A. R., Chi-Rosso, G., Bajardi, A., Getwals, P., Green, C. D., and Kotelniansky, V. E. (2000) Immunity 13, 749–758
12. Hayden, M. S., and Ghosh, S. (2004)
13. Yamamoto, Y., and Gaynor, R. B. (2004)
14. Malek, S., Huxford, T., and Ghosh, G. (1998)
15. Brown, K., Park, S., Kanno, T., Franzoso, G., and Siebenlist, U. (1993)
16. Scott, M. L., Fujita, T., Liou, H. C., Nolan, G. P., and Baltimore, D. (1993)
17. Donath, M. Y., Storling, J., Maedler, K., and Mandrup-Poulsen, T. (2003)
18. Rehman, K. K., Bertera, S., Bottino, R., Balamurugan, A. N., Mai, J. C., Mi, Z., Zeender, E., Maedler, K., Bosco, D., Berney, T., Donath, M. Y., and Halban, P. A. (2000)
19. Herbert, V., Lau, K. S., Gottlieb, C. W., and Bleicher, S. J. (1965)
20. Giannoukakis, N., Rudert, W. A., Trucco, M., and Robbins, P. D. (2003)
21. Cardozo, A. K., Heimberg, H., Heremans, Y., Leeman, R., Kutlu, B., Kruhoffer, M., and Eizirik, D. L. (2001)
22. Pierce, J. W., Schoenleber, R., Jesmok, G., Best, J., Moore, S. A., Collins, T., and Robbins, P. D. (2000)
23. Thompson, J. E., Phillips, R. J., Erdjument-Bromage, H., Tempst, P., and Ghosh, S. (1995) Cell 80, 573–582
24. Parnaud, G., Hammar, E., Rouiller, D. G., and Bosco, D. (2005) Am. J. Physiol. 289, E313–E321
25. Nagata, N., Gu, Y., Hori, H., Balamurugan, A. N., Touma, M., Kawakami, Y., Wang, W., Baba, T. T., Satake, A., Nozawa, M., Tabata, Y., and Inoue, K. (2003) Cell Transplant 12, 447–451
26. Beattie, G. M., Lappi, D. A., Baird, A., and Hayek, A. (1991) J. Clin. Endocrinol. Metab. 73, 93–98
27. Wang, R. N., and Rosenberg, L. (1999) J. Endocrinol. 163, 181–190
28. Thomas, P. T., Contreras, J. L., Bilbao, G., Ricordi, C., Cursel, D., and Thomas, J. M. (1999) Surgery 126, 299–304
29. Kaiser, N., Cores, A. P., Tur-Sina, A., Ariav, Y., and Cerasi, E. (1988) Endocrinology 123, 834–840
30. Kaido, T., Yebra, M., Cirulli, V., and Montgomery, A. M. (2004) J. Biol. Chem. 279, 53762–53769
31. McGivney, L. D., Lu, Z., Bitar, R., Davcek, A. P., Davreux, C. J., and Rotstein, O. D. (1997) J. Biol. Chem. 272, 10285–10294
32. Nahri, N., Lakins, J. N., Russell, A., Ming, W., Chatterjee, C., Rozenberg, G. I., Marinkovich, M. P., and Weaver, V. M. (2003) J. Cell Biol. 163, 1397–1407
33. Norlin, S., Ahlgren, U., and Edlund, H. (2005) Diabetes 54, 125–132
34. Howe, A. K., Aplin, A. E., and Juliano, R. L. (2002) Curr. Opin. Genet. Dev. 12, 30–35
35. Alahari, S. K., Reddig, P. J., and Juliano, R. L. (2002) Int. Rev. Cytol. 220, 145–184
36. Orci, L., Gabbay, K. H., and Malaisse, W. J. (1972) Science 175, 1128–1130
37. Gabbiani, G., Malaisse-Lagae, F., Blondel, B., and Orci, L. (1974) Endocrinology 95, 1630–1635
38. Li, G., Rutherford-Brandle, E., Just, I., Jonas, J. C., Aktories, K., and Wellheim, C. B. (1994) Mol. Biol. Cell 5, 1199–1213
39. Nevins, A. K., and Thurmond, D. C. (2003) Am. J. Physiol. 285, C698–C710
40. Thurmond, D. C., Geissler-Gispert, C., Furukawa, M., Halban, P. A., and Pessin, J. E. (2003) Mol. Endocrinol. 17, 732–742
41. Bernal-Mizrachi, E., Wen, W., Shornick, M., and Permutt, M. A. (2002) Diabetes 51, Suppl. 3, 484–488
42. Furukawa, K., and Mattson, M. P. (1998) J. Neurochem. 70, 1876–1886
43. Papaccio, G., Grazi, A., d’Aquino, R., Valiante, S., and Naro, F. (2005) Am. J. Physiol. 289, C698–C710
44. Chen, L. F., and Greene, W. C. (2004) J. Neurochem. 90, 1630–1635
45. Pessin, J. E. (2004) Mol. Endocrinol. 17, 373–379
46. Amringer, J., Rabeni, C., and Gadekala, C. (2005) J. Biol. Chem. 280, 244–252
47. Rosenberg, L., Wang, R., Paraskar, J., and Battersby, D. (1999) Surgery 126, 393–408
48. Ris, F., Hammar, E., Bosco, D., Pilloud, C., Maedler, K., Donath, M. Y., Oberholzer, J., Zender, E., Morel, P., Rouiller, D., and Halban, P. A. (2002) Diabetologia 45, 841–850