Rapid Publication

Identification of Novel Cytokine-Induced Genes in Pancreatic β-Cells by High-Density Oligonucleotide Arrays

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Type 1 diabetes is an autoimmune disease resulting from the selective destruction of insulin-producing β-cells. Cytokines may contribute to pancreatic β-cell death in type 1 diabetes. β-cell exposure to interleukin (IL)-1β induces functional impairment, whereas β-cell culture for 6–9 days in the presence of IL-1β and interferon (INF)-γ leads to apoptosis. To clarify the mechanisms involved in these effects of cytokines, we studied the general pattern of cytokine-induced gene expression in β-cells. Primary rat β-cells were fluorescence-activated cell sorter–purified and exposed for 6 or 24 h to control condition, IL-1β + INF-γ, or IL-1β alone (24 h only). Gene expression profile was analyzed in duplicate by oligonucleotide arrays. Nearly 3,000 transcripts were detected in controls and cytokine-treated β-cells. Of these, 96 and 147 displayed changes in expression after 6 and 24 h, respectively, of exposure to IL-1β + INF-γ, whereas 105 transcripts were modified after a 24-h exposure to IL-1β. The cytokine-responsive genes were clustered according to their biological functions. The major clusters observed were metabolism, signal transduction, transcription factors, protein synthesis/processing, hormones, and related receptors. These modifications in gene expression may explain some of the cytokine effects in β-cells, such as decreased protein biosynthesis and insulin release. In addition, there was induction of diverse cytokines and chemokines; this suggests that β-cells may contribute to mononuclear cell homing during insulitis. Several of the cytokine-induced genes are potentially regulated by the transcription factor NF-κB. Clarification of the function of the identified cytokine-induced gene patterns may unveil some of the mechanisms involved in β-cell damage and repair in type 1 diabetes. ***Diabetes*** **50:909–920, 2001**

Type 1 diabetes is an autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreatic islets of Langherhans (1). In both human and rodent models of type 1 diabetes, the clinical disease is preceded by a progressive mononuclear cell invasion of the islets (insulitis), which persists for several weeks/months before significant β-cell destruction occurs (2,3). In the course of insulitis, activated macrophages and T-cells secrete soluble mediators such as cytokines, oxygen free radicals, and nitric oxide, which probably all contribute to β-cell dysfunction and death (4–6). Studies in autoimmune diabetes-prone NOD mice and Biobreeding rats indicate that β-cell destructive insulitis is associated with increased expression of proinflammatory type 1 cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (INF)-γ (4–6).

Apoptosis is the main form of β-cell death in NOD mice (7), and there are indications that β-cells also die by apoptosis in the early stages of human type 1 diabetes (8,9). Under in vitro conditions, exposure of human, rat, or mouse purified β-cells to IL-1β, in combination with INF-γ and/or TNF-α, induces severe functional suppression and death by apoptosis (10–14). The prolonged time (6–9 days) required for triggering apoptosis in rodent and human β-cells suggests that de novo gene expression is involved in this process. The identity of the cytokine-affected genes leading to β-cell apoptosis or contributing to β-cell defense/repair remains to be clarified.

Using the "candidate gene" approach (15) and differential display by reverse transcriptase–polymerase chain reaction (RT-PCR) (16), we and other groups (17) have described ~27 genes modified by IL-1β and/or INF-γ. Whereas some of these genes, such as the inducible form of nitric oxide synthase (iNOS) (5), caspase-1 (18), cyclooxygenase (COX)-2 (19), and macrophage chemoattractant protein (MCP)-1 (16), are potentially related to insulitis and β-cell damage, others, such as magnanese

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AS, argininosuccinate synthase; CINC, cytokine-induced neutrophyl chemoattractant; CKK, cholecystokinin; COX, cyclooxygenase; ERK, extracellular signal related kinase; EST, expression sequence tag; FACS, fluorescence-activated cell sorter; GABA, γ-aminobutiric acid; GADD, growth arrest and DNA damage; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GH, growth hormone; GIP, gastric inhibitory peptide; HO, heme oxygenase; hsp, heat shock protein; ICAM, intracellular adhesion molecule; IL, interleukin; INF, interferon; iNOS, inducible nitric oxide synthase; IRF, interferon regulatory factor; MAPK, mitogen-activated protein kinase; MCP, macrophage chemoattractant protein; MGMT, *O*-6 methylguanine-DNA methyltransferase; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; MnSOD, magnanese superoxide dismutase; NF, nuclear factor; Pdx-1, pancreatic duodenal homeobox factor-1; PRL, prolactin; RT-PCR, reverse transcriptase–polymerase chain reaction; TNF, tumor necrosis factor; VAMP, vesicle-associated membrane protein.

superoxide dismutase (MnSOD) (20), heme oxygenase (HO)-1 (21), heat shock protein (hsp)-70 (22), and A20 (23), are probably part of β-cell defense mechanisms. Unfortunately, none of these proteins seems to be the decisive factor for cytokine-induced apoptosis. It is thus conceivable that β-cell fate after immune-mediated damage will depend on an intricate pattern of dozens of genes up- or downregulated parallel and/or sequentially and not on single genes. Identification of a complex pattern of gene expression is now feasible by the use of high-density oligonucleotide probe arrays (24–26).

To identify early and late genes involved in cytokine-induced β-cell dysfunction and death or in defense/repair, we presently carried out expression profile of fluorescence-activated cell sorter (FACS)–purified rat β-cells exposed for 6 and 24 h to a combination of IL-1β + INF-γ or IL-1β alone (24 h). Whereas IL-1β + INF-γ leads to β-cell apoptosis (10–14), IL-1β alone induces β-cell functional suppression but not cell death (27,28).

RESEARCH DESIGN AND METHODS

Islet cell isolation and culture and nitrite measurement. Pancreatic islets were isolated from male Wistar rats 10 weeks of age by collagenase digestion, and islet β-cells were purified by autofluorescence-activated cell sorting (29) (FACStar, Becton-Dickinson, Sunnyvale, CA). β-cell preparations were cultured at 37°C as aggregates in suspension in Ham's F10 medium (Gibco Brl-Life Technologies, Paisley, U.K.), as previously described (30). For the microarray analysis, purified rat β-cells were precultured in Ham's medium for 16 h and then exposed to the following conditions: control for 6 h (no cytokines added), IL-1β + INF-γ for 6 h, control for 24 h, IL-1β + INF-γ for 24 h, and IL-1β for 24 h. The number of experimental conditions tested (5) was adapted to the number of microarrays present in the Affymetrix package (5). For the RT-PCR confirmation experiments, four groups were studied (control, IL-1β, INF-γ, and IL-1β + INF-γ conditions), and all groups were studied at both 6 and 24 h. IL-1β (tested at 50 U/ml, 38 U/ng) was a kind gift from Dr. C.W. Reinolds from the National Cancer Institute, Bethesda, MD, and INF-γ (tested at 1,000 U/ml, 10 U/ng) was purchased from Holland Biotechnology, Leiden, the Netherlands. The choice of cytokine concentration and the time of exposure was based on our previous data (10,13,14,16) and aimed to identify genes that are either directly induced by the cytokine(s) and/or result from β-cell responses to cellular stress (mostly after 24 h). After a 24-h exposure to INF-γ + IL-1β, most β-cells are still viable, but ~10% of the β-cell population is already committed to undergo apoptosis. However, the morphological changes of cell death (concomitant to nonspecific changes in gene expression) are only apparent after a subsequent 48- to 72-h culture (A.K.C. and D.L.E., data not shown). Culture media from the cells used for the microarray analysis were collected after 24 h for nitrite determination (nitrite is a stable product of NO oxidation), as previously described (31).

Microarray analysis. For the microarray analysis, samples of control and cytokine-treated cells were harvested, and total RNA was isolated using RNeasy kit (Qiagen). Because it was difficult to obtain a sufficient number of rat β-cells in a single occasion, and in order to decrease eventual biases due to biological variation, the cells were pooled from six separated experiments, using 2.5×10^5 cells per group in each experiment. Ten micrograms total RNA were obtained from each pooled experimental group, and the RNA was converted into double-stranded cDNA using a cDNA synthesis kit (Superscript Choice; Gibco, Gaithersburg, MD) with a special oligo(dT)24 primer containing a T7 RNA promoter site added 3′ of the poly-T tract. Biotinylated cRNAs were generated from purified cDNAs using the Bioarray high-yield RNA transcript labeling kit (Enzo Diagnostics, Farmingdale, NY). The cRNA sample was purified with the RNeasy kit (Qiagen), and 20 μg of each cRNA sample was prepared and hybridized as previously described (32). Analysis of differential expression was performed by GeneChip software (version 3.3). Normalization was performed by global scaling, with the arrays scaled to an average intensity of 150. Duplicate hybridizations using separate sets of chips were performed for all conditions. Cytokine-induced differences in gene expression were considered present when the fold-change was ≥3.0 in both experiments. In case genes were previously described as modified by cytokines by other techniques or present in more than one experimental condition (with values >3 in at least one condition), we considered changes ≥2.0 sufficient for inclusion in Table 1. Note that the GeneChip software allocates an arbitrary value of 20 for genes below detection limit. This is done to allow calculation of the gene expression in the experimental group as the fold variation compared with the reference group. Thus, in cases in which the reference genes were undetectable and the allocated fold-variation was between 3 and 10, we indicated the values as >3.0 or <–3.0. If the allocated variation was >10, we indicated it as >10 or <–10.0 (Table 1). Genes were classified on different functional clusters (Table 1) based on the putative biological function of the encoded protein, as determined by database searches on PubMed, gene cards from the Weizmann Institute of Science (http://bioinfo.weizmann.ac.il/bioinfo.html), and a previously published classification scheme for cellular functions (33).

Note that our β-cell preparations have a purity of ~95%, but also contain 1–4% of α-cells, δ-cells, and PP-cells (29). We observed the presence of both glucagon, somatostastin, peptide YY, and prepropancreatic polypeptide mRNAs among the genes detected by the present analysis (data not shown). Thus, it cannot be excluded that a minor proportion of the presently observed changes in gene expression occurred in these other cell types.

mRNA isolation and RT-PCR. RT-PCR using specific primers was performed to confirm the differential expression of 17 mRNAs detected with the microarray analysis. The selection of RT-PCR instead of Northern blot analysis was motivated by the limited availability of primary β-cells. mRNA isolation and RT-PCR were performed as previously described (16). The number of cycles was selected to allow linear amplification of the cDNA under study. For semiquantitative PCR, the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as control. We have previously shown (34) and confirmed in these experiments (data not shown) that IL-1β and INF-γ do not affect GAPDH mRNA expression in insulin-producing cells. The primers sequences and their respective PCR fragment lengths were as follows: GAPDH F 5′-TCCCTCAAGATTGTCAGCAA-3′, R 5′-AGATCCA-CAAACGGATACATT-3′ (308 bp); iNOS F 5′-GACTGCACAGAATGTTCCAG-3′, R 5′-TGGCCAGATGTTCCTCTATT-3′ (308 bp); thyrotropin-releasing hormone F 5′-CCTAACTGGTATCCCTGAAT-3′, R 5′-TGAGAACCAGGAATCCAGAA-3′ (392 bp); Isl-1 F 5′-TTTGGACGAAAGCTGTACCTG-3′, R 5′-TGGATGCAAGG-GACTGAGAG-5′ (304 bp); mob-1 F 5′-TGAGTCTGAGTGGGACTCAA-3′, R 5′-CCTTGCTGCTGGAGTTACTT-3′ (452 bp); growth arrest and DNA damage (GADD)-153: F 5′-CTTTGAGACAGTGTCCAGCT-3′, R 5′-TTCAGCAAGC-TGTGCCACTT-3′ (377 bp); lactate dehydrogenase *B* F 5′-ACTCCGTGA-CAGCCAATTCT-3′, R 5′-AGCATGGATTCGATGAGGTC-3′ (543 bp); gastric inhibitory peptide (GIP) receptor F 5′-ATGTGCCTGGAGATGAGGTC-3′, R 5′-ACCTGCATTCCTCTCACTGG-3′ (541 bp); GLUT1 F 5′-AACCTGTTGGC-CTTTGTGTC-3′, R 5′-ATCTGCCGACCCTCTTCTTT-3′ (454 bp); IL-15-F 5′-AAAGAGGAGGCGTTCTGGAT-3′, R 5′-GCTGTTTGCAAGGTAGAGCA-3′ (452 bp); prolactin receptor (PRL) F 5′-CAAATGGGAAGCAGTTCCTC-3′, R 5′-CCACTGCCCAGACCATAATC-3′ (448 bp); growth hormone (GH) receptor F 5′-ATGAGCCCGATATGGTCAAC-3′, R 5′-TGGTACGTCCAGAATCGTCA-3′ (547 bp); Janus protein tyrosine kinase-2 (JAK-2) F 5′-CTGCAGGACAACACT-GGAGA-3′, R 5′-GGGACTTTCACCTGGTTCCT-3′ (441 bp); nuclear factor (NF)-κB-p105 F 5′-TCATCCACCTTCATGCTCAG-3′, R 5′-GCCAACGAGAT-GTTGTCGTA-3′ (447 bp); C/EBPδ F 5′-GAGACTCCGAACGACCGATA-3′, R 5′-GTCAGAGCTGGTGCCTCTTT-3′ (310 bp); osteoprotegerin F 5′-CGCATCT-TGATGGAGAGCTT-3′, R CATTTCAAGAGCCGGAGAAG-3′ (402 bp); macrophage inflammatory protein (MIP)-3α F 5′-TGAGAATGGCCTGCAAGCAT-3′, R 5′-TCCATTGGACAAGACCACTG-3′ (425 bp); and fractalkine F 5′-CAATCT-GGATCTAGCCTCTG-3′, R 5′-AGTCGGGACAGGAGTGATA63′ (379 bp). The abundance of the PCR products of interest was expressed in pixel intensities (optical density [OD]), normalized using the maximum signal in each amplification as 10, and divided for the abundance of the GAPDH signal amplified in parallel from the same cDNA sample. The data are presented as a percentage of the respective controls. When control values were below detection limits, the data were presented as their normalized pixel intensities.

Statistical analysis. Data are presented as means ± SE, and comparisons between groups were performed by Student's paired *t* test.

RESULTS AND DISCUSSION

To identify early and late cytokine-induced genes in pancreatic β-cells, these cells were exposed to the following treatments: control condition for 6 h, IL-1β + INF-γ for 6 h, control condition for 24 h, IL-1β + INF-γ for 24 h, and IL-1β for 24 h. After a 24-h culture, there was no detectable nitrite production by the control (non–cytokine-exposed) β-cells. On the other hand, IL-1β–treated cells released (mean ± SE) 7.0 ± 1.1 pmol nitrite $\cdot 10^{-3}$ cells $\cdot$ 24 h^{-1}, whereas cells exposed to IL-1β + INF-γ released 13.6 ± 1.5 pmol nitrite $\cdot 10^{-3}$ cells $\cdot$ 24 h^{-1} ($n = 6$; $P < 0.001$ vs.

TABLE 1
Modifications in β-cell gene expression after cytokine exposure

Cluster/GAN	Gene name	IL-1β + IFN-γ				IL-1β	
		6 h		24 h		24 h	
		H1	H2	H1	H2	H1	H2
1.0 Metabolism							
1.1 Carbohydrates							
X59737*	Creatine kinase-ubiquitous	>+3.0	>+10.0	>+3.0	>+3.0	>+3.0	>+3.0
S68135*	GLUT1			+3.6	+3.8	+3.5	+4.3
L28135	GLUT2			–4.5	–3.7		
X53588	Glucokinase			–4.9	–4.2	–4.7	–3.5
U07181*	Lactate dehydrogenase-B	–4.3	–5.9				
1.2 Arginine metabolism and NO formation							
U03699*	iNOS	>+10.0	>+10.0	>+10.0	>+10.0	>+3.0	>+3.0
X12459	Argininosuccinate synthetase	>+3.0	>+10.0	>+10.0	>+10.0	>+3.0	>+10.0
J04792*	Ornithine decarboxilase			+3.5	+2.4	+5.4	+5.6
J02720	Arginase	–2.5	–2.5	–2.1	–5.0	–5.3	–2.7
1.3 Amino acids (other than arginine)							
M72422	GAD65	–6.4	–3.3	–5.8	–4.1	–5.3	–2.3
U91561	Pyridoxine 5′-phosphate oxidase	<–3.0	<–3.0	–3.0	–2.2	–3.1	–2.4
M96601	Taurine transporter			–3.1	–6.3		
Z15123	S-adenosylmethionine decarboxylase	<3.0	–3.3	–2.0	–3.9		
E03229	L-cysteine oxygen oxidoreductase	<–3.0	<–3.0	–2.5	<–3.0		
M97662	β-alanine synthase			–3.4	–2.9	–6.0	–4.7
M84648	L-amino acid decarboxilase			<–3.0	<–10.0	<–3.0	<–10.0
1.4 Lipids							
S69874	c-FABP		+8.9	+9.5	+10.8	+9.8	
D17309	Δ-4-3-ketosteroid 5-β reductase			+4.6	+5.2	+3.3	+4.8
AF048687	Lactosylceramide synthase			–3.6	–3.3		
J05035	Steroid 5 α-reductase			<–3.0	–2.4	<–3.0	<–3.0
L27075	ATP-cytrate lyase			–2.2	–2.3	–3.8	–4.3
S70011	Trycarboxilate carrier-mithocondrial	–4.8	–2.8	–2.3	–2.8		
U44750	15-PGDH	–2.7	–3.5	<–3.0	<–3.0	<–3.0	<–3.0
M73714	Microsomal aldehyde dehydrogenase	<–3.0	<–10.0	<–3.0	<–3.0		
J05470	Carnitine palmitoyltransferase II	<–10.0	–2.4	<–10.0	–6.4	<–3.0	–2.3
D00569*	2,4-dyenoyl-CoA reductase	–4.3	–5.4	<–3.0	<–10.0	<–3.0	–2.3
1.5 ATP production and processing							
U78977*	ATPase (putative)			–4.5	–3.9	–3.7	–3.1
D00636*	Cytochrome b-5 reductase (NADH)	<–10.0	<–3.0	<–10.0	<–10.0	<–3.0	<–10.0
1.6 Miscellaneous							
J05519	C1-tetrahydrofolate synthase	+4.1	+7.3	>+3.0	>+3.0		
D87839	GABA transaminase	–4.1	–3.1				
M83143*	β-galactoside α-2,6-sialyltransferase	–3.0	–3.7	–8.4	–6.8	<–3.0	–2.7
2.0 Protein synthesis, modification, and secretion							
AJ000485	CLIP 115	>+3.0	>+3.0	>+3.0	>+3.0		
X00722	32S pre-rRNA	+2.9	+2.7	>+3.0	>+3.0		
J03627	S-100–related protein	+4.1	+3.6	+2.9	+3.8		
X77235	ARL 4	+3.0	+4.5	>+3.0	+2.9		
M24105	VAMP2			–2.7	–2.6	–3.1	–2.4
X06889	Rab 3A	<–3.0	<–3.0	<–3.0	–2.6		
M83745	Prohormone convertase-1			–3.8	–3.2		
X53565	TGN 38	–2.3	–3.3	<–3.0	–2.6	<–3.0	–7.2
M75148	Kinesin light chain-C	<–3.0	<–3.0				
U72995	Rab 3 GDP/GTP exchange protein			<–3.0	<–10.0	<–3.0	<–3.0
M96630	Sec 61 homolog	<–3.0	–2.6	–2.8	<–10.0	<–3.0	<–3.0
3.0 Ionic channels and ion transporters							
X96394	Multidrug resistance protein			>+3.0	+2.5	+3.4	+3.9
AF008439	Nramp2	+4.6	+4.1	+2.9	+2.5	+4.4	+7.8
M58040	Transferrin receptor			>+3.0	>+3.0		
AF004017	Eletrogenic NA + bicarbonate cotransporter	>+3.0	>+3.0				
AF048828*	RVDAC 1					–3.4	–3.2
U08290	Neuronatin α			–2.4	–2.5	–3.9	–3.4
U50842	Nedd4 ubiquitin ligase			–3.8	–3.7	–2.7	–1.9
J04024*	Ca^{2+} ATPase type 2	<–3.0	<–3.0	–2.3	<–3.0		

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TABLE 1
Continued

Cluster/GAN	Gene name	IL-1β + IFN-γ 6 h H1	IL-1β + IFN-γ 6 h H2	IL-1β + IFN-γ 24 h H1	IL-1β + IFN-γ 24 h H2	IL-1β 24 h H1	IL-1β 24 h H2
4.0 Hormones and growth factors							
M11596	Calcitonin-related peptide, β	+7.3	+9.7	+9.6	+11.0	+5.4	+6.1
X63574	Somatostatin receptor type 3	>+3.0	>+10.0	>+3.0	+3.4	>+3.0	+4.1
M25804*	Rev-ErbA-α			+2.6	+3.7	+3.5	+3.7
D15069	Adrenomedullin precursor	+3.8	+3.7	+2.8	+3.2		
M96674	Glucagon receptor	>+3.0	>+3.0	+3.2	+2.1	+4.9	+3.3
Z83757	GH receptor	<–3.0	–7.5	–2.3	<–3.0		
AA818097	Glucagon-like peptide 1 receptor			–3.2	–4.2	–3.4	–2.0
M25584	Insulin 1			–3.0	–4.3	–3.1	–2.1
M93273	Somatostatin receptor type 2	<–3.0	<–10.0	–4.2	–4.5	–4.3	–6.7
L19660	Gastric inhibitory peptide receptor			–4.7	–5.1	–4.1	–3.7
M74152*	Prolactin receptor			–5.5	–4.7	–3.7	–2.7
M36317*	Thyrotropin releasing hormone			–5.3	–5.3	–3.7	–3.7
D50608*	Cholecystokinin-A receptor	<–10.0	–8.5	<–10.0	<–10.0	<–10.0	–21.0
5.0 Cytokines, chemokines, and related receptors							
D11445	CINC-1	>+10.0	>+10.0	>+10.0	>+10.0	>+10.0	>+10.0
U17035	Mob-1	>+10.0	>+10.0	>+10.0	>+10.0	>+3.0	>+3.0
X17053*	MCP-1	>+10.0	>+10.0	>+10.0	>+10.0	>+3.0	>+3.0
AF053312	MIP-3α	>+10.0	>+10.0	>+3.0	>+10.0	>+3.0	>+10.0
AF030358*	Fractalkine	>+10.0	>+10.0				
U94330	Osteoprotegerin	>+10.0	>+10.0				
AJ011969	MIC-1			>+3.0	>+3.0		
U45965	CINC-3	>+10.0	>+10.0	>+3.0	>+3.0		
U69272*	IL-15	>+10.0	>+10.0	>+3.0	>+3.0		
M26744	IL-6	>+3.0	>+3.0	>+3.0	>+3.0		
6.0 Cytokine processing and signal transduction							
M80367	Guanylate nucleotide binding protein 2	>+10.0	>+10.0	>+10.0	>+10.0		
AJ000557*	JAK-2	>+3.0	>+10.0				
AF086624	Pim-3 serine threonine kinase			>+10.0	>+3.0	>+3.0	>+10.0
D89863	M-Ras	>+3.0	+15.2	>+3.0	+7.4		
S81478*	3CH134/CL100 tyrosine phosphatase	>+3.0	+3.5	>+3.0	>+3.0		
M64301*	ERK3	+3.3	+4.3	+3.2	+1.7	+2.4	+2.4
M64780*	Agrin	+5.1	+6.2	+3.3	+2.9		
AA957896	MAPK kinase 2			–3.3	–3.4	–3.1	–2.5
X85183	Ras-related GTPase (rag A)			–3.8	–3.4	–2.4	–2.5
X74227	IP3 3-kinase	<–3.0	<–3.0				
J05592*	Phosphatase inhibitor-1	–4.2	–2.9	–3.6	–4.8	–3.1	–2.4
U22830	P2Y purinoreceptor			–4.4	–5.0	<–3.0	<–3.0
M85214	Tyrosine kinase receptor			–6.8	–5.1	–4.8	–3.7
M62372	α-2-adrenergic receptor (RG 20)	<–3.0	<–10.0	<–3.0	<–3.0	<–3.0	<–3.0
M23601	Monoamine oxidase B			–7.3	–9.0	–5.6	–4.1
AF013144	MAPK phosphatase (cpg21)	–3.3	–3.7	–9.8	–10.8	–3.1	–4.5
7.0 MHC and related genes							
X57523*	Mtp1	>+10.0	>+10.0	>+10.0	>+10.0	>+3.0	>+10.0
D10729	Proteasome subunit RC1	>+3.0	>+10.0	>+10.0	>+10.0	>+3.0	>+10.0
X14254	MHC-II–assoc. invariant chain γ	>+3.0	>+10.0	>+10.0	>+10.0		
X67504	MHC-I molecules			>+10.0	>+10.0		
M64795	MHC-I RT1-u haplotype	>+3.0	>+10.0	>+10.0	+7.9		
AF029240*	MHC-1b RT1.S3	>+10.0	>+10.0	+9.6	+16.8		
D10757*	Proteasome subunit RING 12	>+10.0	>+10.0	>+3.0	>+10.0		
M31038	MHC-I non-RT1.α chain	+6.9	+4.9	+8.4	+8.5		
U16025*	MHC-Ib RT1	>+3.0	>+10.0	>+3.0	>+3.0		
M10094	MHC-I truncated cell surface antigen	+3.6	+6.7	>+3.0	+3.8		
D30804*	Proteasome subunit RC6-1			+4.2	+4.9		
X63854	Mtp2	+8.0	+12.8	+3.2	+4.9		
M15562*	MHC-II RT1.u-D-α chain			+4.4	+3.1		
D45250*	Proteasome activator rPA28-β	+5.8	+3.8	+3.3	+4.1		
8.0 Cell adhesion, cytoskeleton, and related genes							
X81449*	Keratin 19	>+10.0	>+10.0	+7.7	+7.0	+7.3	+7.7
U05675	Fibrinogen β	+4.9	+5.7	+4.5	+6.9	+6.9	+9.0
D00913	ICAM-1	>+10.0	>+10.0	>+3.0	>+3.0	>+3.0	>+3.0
AF017437*	CD 47 antigen	+3.4	+3.2	+3.8	+4.0	+2.6	+2.4

Continued on following page

TABLE 1
Continued

Cluster/GAN	Gene name	IL-1β + IFN-γ				IL-1β	
		6 h		24 h		24 h	
		H1	H2	H1	H2	H1	H2
M23697	Tissue-type plasminogen activator			+3.2	3.7	+6.6	+5.7
U49062*	Antigen CD-24			–3.5	–3.2	–4.2	–3.3
X05834*	Fibronectin			–7.0	–3.9	–5.2	–4.7
AA875659*	Internexin-α	–2.7	<–3.0	<–3.0	<–10.0		
D83348	Long-type PB-cadherin			<–3.0	<–10.0	<–3.0	–3.5
9.0 Transcription factors and related genes							
X63594*	I-κB α-chain	>+10.0	>+10.0	>+10.0	>+10.0	>+10.0	>+10.0
AF001417	Zinc finger protein 9	>+10.0	>+10.0	>+10.0	>+10.0	>+3.0	>+10.0
Y00396*	c-*myc*			+6.2	+4.1	+7.4	+5.0
X17163	c-jun	>+3.0	>+10.0	>+3.0	>+10.0		
M34253*	IRF-1	+8.3	+12.4	+9.4	+12.4	+2.2	+2.2
S71523	Lim-1					>+3.0	>+10.0
J03179*	D-binding protein			+5.0	+3.0	+6.5	+4.5
L26267	NF-κB–p105	+6.1	+8.8	+8.3	+4.4	+5.0	+4.9
AF031657	Zinc finger protein 94					>+3.0	>+3.0
X62323	Pan-1					>+3.0	>+3.0
M65149*	C/EBPδ	+2.6	+3.0	+3.5	+3.7	+4.2	+4.1
S77528*	C/EBPβ	+2.5	+2.0	+3.0	+3.4	+2.7	+3.6
S66024*	CREM transcriptional repressor	–2.6	–3.1	–5.1	–2.9	–6.5	–6.2
U08214	URE-B1 DNA binding protein			–3.8	–5.5		
U04835	CREM δ C-G	<–3.0	–2.2	<–3.0	–2.9	<–3.0	–2.5
AA900476*	MRG 1	–3.5	–3.5	–2.6	–3.0	–3.4	–2.3
S69329*	Isl-1	–2.5	–2.3	–6.3	–3.5	–3.0	–2.6
U67080	Zinc finger protein MyT13			–5.0	–11.5	–3.4	–4.5
10.0 RNA synthesis and splicing factors							
AF063447	RNA helicase			>+3.0	+3.6	>+3.0	+4.4
AF044910	Survival motor neuron					>+3.0	>+3.0
AF036335*	NonO/p54nrb	–3.3	–3.3				
11.0 Cell cycle							
U75404*	SSeCKs 322	+7.6	+6.6				
D14014	Cyclin D1	–3.5	–2.7	–3.1	–2.1	–3.3	–2.2
D16308	Cyclin D2			–3.1	–3.7	–2.7	–1.8
AA874802	Histone H1 subtype O			–3.1	–4.7	–5.4	–5.8
D16309*	Cyclin D3	<–3.0	–2.9	<–3.0	<–3.0	<–3.0	–2.9
12.0 Defense/repair							
M85389*	Hsp 27			>+10.0	>+10.0		
AA859648	Hsp 40-mouse homolog			+7.6	+6.0	+2.7	+2.5
Z27118*	Hsp 70-gene 1/2			+7.1	+5.3		
U30186	GADD-153			+3.6	+4.3	+4.9	+6.6
AA875620*	Hsp 70-gene 3			+3.7	+3.3	+4.6	+4.1
M76704	MGMT			>+3.0	+2.5	>+3.0	+2.9
M11794	Metallothionein			+2.0	+4.8	+2.1	+3.3
U77829	Gas-5 growth arrest homolog			+3.7	+3.1	+2.1	+3.3
Y00497	MnSOD	+3.1	+3.8	+2.4	+4.1	+2.4	+3.9
X52711	MX1	>+3.0	>+3.0	>+3.0	>+3.0		
D00680	Glutathione peroxidase			<–3.0	<–3.0	<–3.0	<–3.0
D42148	Gas-6 growth arrest specific	<–3.0	<–3.0	<–3.0	<–10.0	–2.9	<–3.0
13.0 Apoptosis							
E13573	Death protein-5			>+3.0	>+10.0	>+3.0	>+3.0
14.0 Miscellaneous							
Y0704*	Best-5	>+10.0	>+10.0	>+10.0	>+10.0		
J02962	IgE binding protein	+3.5	+2.8	+8.7	+11.0	+9.6	+10.2
M29866*	Complement component-3	>+3.0	>+10.0	>+3.0	>+10.0	>+3.0	>+10.0
D88250	Complement C1 homologue	>+3.0	>+10.0	>+3.0	>+10.0		
AA875037	Serine protease inhibitor-15			>+3.0	>+3.0		
S45663	Sinaptic glicoprotein-2			+4.7	+4.2	+4.0	+4.1
S75019*	Antiquitin			–3.2	–3.3	–2.6	–2.3
U10071	CART			–5.1	–4.2	–3.4	–3.8
D10666	Neural visinin-like protein 1	–2.1	–5.4	–4.7	–5.5	–4.7	–4.7
AF080468*	Glycogen storage disease-1b protein	–3.8	–3.2	–5.6	–5.2	–3.7	–2.8
U64030	Deoxyuridine triphosphatase	<–3.0	–4.6	<–3.0	–5.8	–2.3	–2.8

Data are fold-variation for the gene with the indicated access number. In some cases, the fold-change was arbitrarily estimated by the software due to undetectable expression in one of the groups being studied (see RESEARCH DESIGN AND METHODS). In these cases, when the allocated fold-variation was between 3 and 10, it is indicated as >+3.0 or <–3.0. If the allocated variation was >10, we indicated it as >+10 or <–10. The genes are ordered taking into account the fold-variation in gene expression of the cells exposed for 24 h to IL-1β + INFγ. The data are from individual duplicate hybridizations (H)-1 and H2. *Different expression of a gene detected by more than one group of probes. +, increased; –, decreased compared with respective controls (β-cells not exposed to cytokines; 6 or 24 h). ARL4, ADP-ribosylation–like 4; CART, cocaine- and amphetamine-regulated transcript; c-FABP, cutaneous fatty acid binding protein; CREM, cAMP-responsive element modulator; GAN, GenBank accession number; RvDAL, voltage-dependent anion channel; TGN, Trans-Golgi network integral membrane protein.

cells exposed to IL-1β alone). These results are similar to our previous observations (5,35) and confirm that both IL-1β and INF-γ were biologically active.

Cells from the six separate experiments described above were pooled for RNA extraction, and the resulting biotinylated cRNAs were hybridized in duplicate to the Affymetrix rat U34-A oligonucleotide array containing ~8,000 probes (77% known genes and 23% expression sequence tags [ESTs]). Approximately 3,000 genes or ESTs were scored as present in each of the five conditions (2,700– 3,300). The cytokine-modified known genes are shown in Table 1.

Scatter plot analysis comparing expression levels in control and cytokine-exposed β-cells at both 6 and 24 h showed a large number of cytokine-responsive genes (data not shown). After a 6-h exposure of β-cells to IL-1β + INF-γ, 96 known genes were differentially expressed (Table 1). Exposure of β-cells to the same combination of cytokines for 24 h modified the expression of 147 known genes (Table 1). IL-1β alone induced the differential expression of 105 known genes (Table 1).

To validate the microarray results, we initially compared the data with the available information on cytokine-induced gene expression (15,17). Approximately 27 genes and/or proteins have been previously described as modified by IL-1β and/or INF-γ in whole islets or β-cells using a time schedule similar to the one presently used (e.g., 6- and 24-h exposure to cytokines). Of these 27 genes, 22 were detected in the present analysis (80%). These genes are insulin (decreased), iNOS (increased), arginase (decreased), argininosuccinate synthase (AS) (increased), ornithine decarboxilase (increased), hsp-70 (increased), MnSOD (increased), HO-1 (increased), intracellular adhesion molecule (ICAM)-1 (increased), glucokinase (decreased), GLUT2 (decreased), prohormone convertase-1 (decreased), GAD65 (decreased), MCP-1 (increased), cytokine-induced neutrophyl chemoattractant (CINC)-1 (increased), CINC-3 (increased), major histocompatibility complex (MHC) class I (increased), interferon regulatory factor (IRF)-1 (increased), c-jun (increased), adenine nucleotide translocator-1 (increased), COX-2 (increased), and phospholipase-D1 (decreased). Of the five genes not detected in the present analysis, as modified by cytokines, two genes (pancreatic duodenal homeobox-1 [Pdx-1] and serine protease inhibitor-3) were not present in the array, whereas three genes were present in the array but were either considered as below detection limit (Fas and caspase-1), or were detected but considered "no change" after cytokine exposure (prohormone convertase-2).

To further validate the results of the microarray analysis, we selected 17 genes for confirmation by RT-PCR (Fig. 1). Induction of iNOS, a well-known effect of cytokines in β-cells (15), was used as a positive control. As previously described (15), IL-1β and IL-1β + INF-γ induced a high iNOS expression after 6 h, with a subsequent decline after 24 h of continuous exposure to the cytokines (Fig. 1). All 17 genes considered "changed" by the microarray analysis were confirmed by the RT-PCR as modified by cytokines. The RT-PCR analysis and the comparisons with genes and proteins previously described as modified by cytokines (see above) confirm that microarray analysis, performed in duplicate using pooled cells from several experiments, is a reliable method to detect massive variations in β-cell mRNA expression. Similar conclusions were found in a recent study in which microarray analysis was performed to determine glucose regulation of secretory and metabolic pathway genes in MIN6 insulin-producing cells (36). Note that microarrays are a relatively new technique; the present study, for instance, is the first in which this method is applied for characterization of gene expression in primary β-cells. Ideally, all genes described as modified by the microarray analysis should be confirmed by additional techniques, such as RT-PCR or Northern blot. Thus, the presently described modifications in mRNA expression, which were not confirmed by RT-PCR, should be viewed with caution.

The cytokine-responsive genes were clustered according to the putative biological function of their encoded proteins, as indicated in Table 1. The most frequent changes were observed in β-cell metabolism, with 19, 20, and 20% of all differentially expressed genes induced, respectively, by IL-1β + INF-γ for 6 h, IL-1β + INF-γ for 24 h, and IL-1β alone for 24 h. IL-1β + INF-γ induced an early suppression of β-cell metabolism, with a decrease in ~80% of the 18 modified genes after 6 h exposure (Table 1, item 1.0). This suppression was maintained at 24 h, with nearly 70% of the metabolism-related genes inhibited by IL-1β + INF-γ or IL-1β alone. These alterations occurred in genes related to the metabolism of carbohydrates, amino acids (others than arginine), lipids, and ATP production.

Among the carbohydrate-related genes, it is noteworthy that cytokines decreased the expression of mRNAs for GLUT2 and glucokinase, whereas they increased expression of GLUT1 (Table 1, item 1.1). IL-1β–induced decreases in GLUT2 have been previously shown at the protein level (37), whereas the present microarray results on GLUT1 were confirmed by RT-PCR (Fig. 1). GLUT2-null knockout mice are hypoinsulinemic and hyperglycemic, but they regain normal insulin secretion and glycemia after transgenic expression of GLUT1 in β-cells (38). Thus, the presently observed upregulation of GLUT1 in cytokine-treated β-cells may represent an adaptive/compensatory mechanism for the decrease in GLUT2 expression. This compensatory response seems to be effective, i.e., rat islets exposed to IL-1β or IL-1β + TNF-α preserve normal glucose utilization but have defective glucose oxidation (39), probably caused by NO-induced aconitase blocking (40).

After a 24-h exposure to IL-1β or IL-1β + INF-γ, there were modifications in the expression of three key genes related to arginine metabolism and nitric oxide formation (Table 1, item 1.2). Thus, there was a parallel induction of iNOS and AS expression at 6 and 24 h, whereas arginase was inhibited. iNOS uses arginine as substrate for NO production, generating citrulline as a byproduct. Citrulline can be recycled into arginine by AS activity (41,42). This and the concomitant inhibition of arginase, an enzyme responsible for arginine degradation (43), will allow a continued arginine supply for NO production by the β-cells.

One of the well-known effects of cytokines in β-cells is inhibition of insulin mRNA expression (confirmed in the present array analysis), total protein and pro-insulin biosynthesis, and decreased insulin release (4,5,44). We presently observed downregulation after both 6- and 24-h exposures to cytokines of several genes related to protein synthesis, modification, and secretion (Table 1, item 2.0),

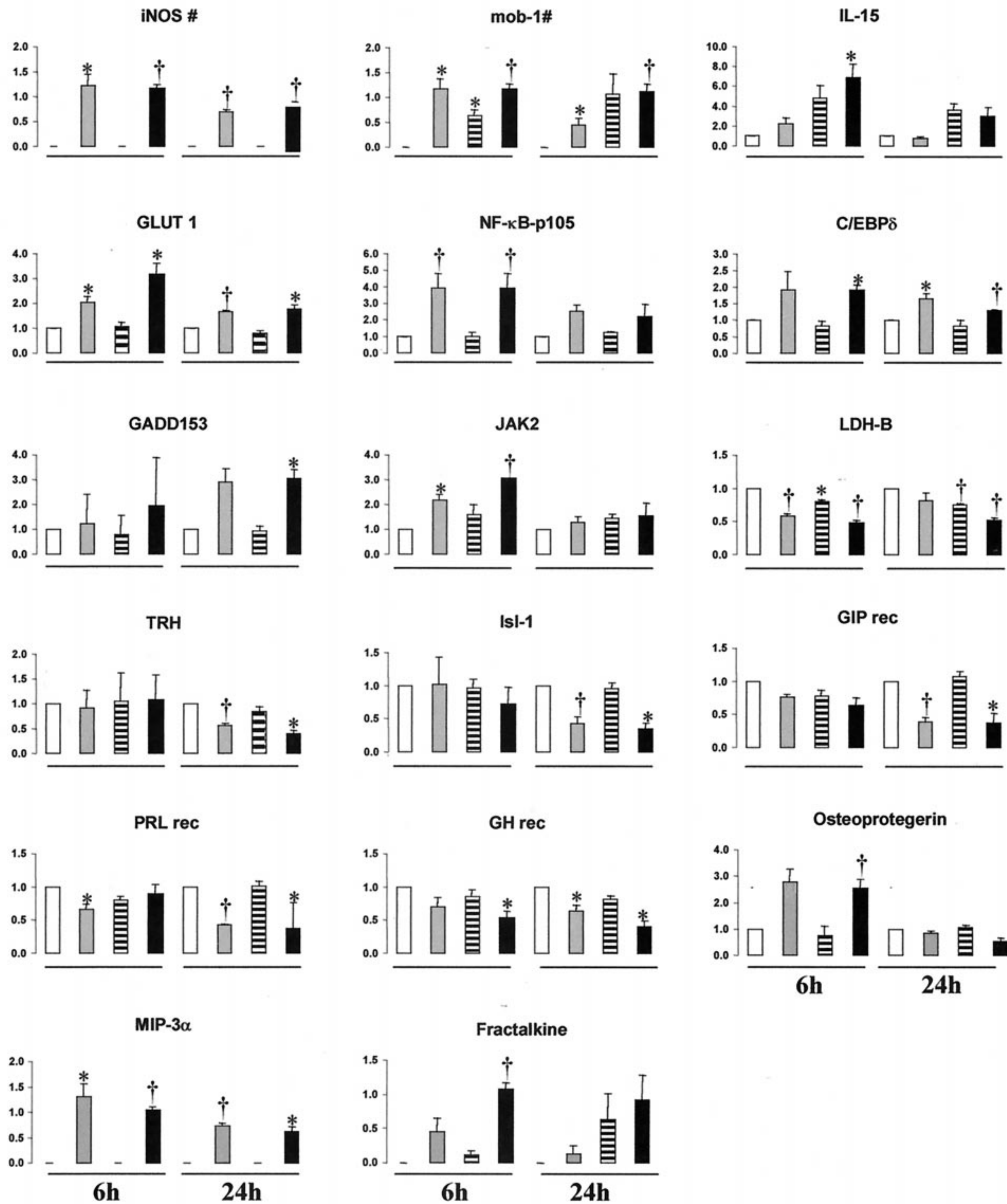

FIG. 1. Confirmation by RT-PCR of the genes detected by duplicated microarray analysis as modified by cytokines in purified rat β-cells (Table 1). Rat β-cells (10^5 cells/condition) were exposed for 6 or 24 h to the following conditions: control (no cytokine added) (open bars), IL-1β (50 U/ml) (shaded bars), INF-γ (1,000 U/ml) (striped bars), and IL-1β + INF-γ (filled bars). After these time points, the cells were harvested, mRNA extracted, and RT-PCR performed with the equivalent of 1.5×10^3 cells. PCR band intensities were expressed as OD corrected for GAPDH expression. The data are presented as a percentage of the respective controls, which received an arbitrary value of 1 in each experiment. #When control values were not detectable, the absolute OD values corrected by GAPDH are presented. Data are means ± SE of 3–5 experiments. LDH-B, lactate dehydrogenase B; rec, receptor. *$P < 0.05$; †$P < 0.01$ vs. corresponding control groups, paired Student's *t* test.

which may at least partly explain the above described decrease in protein synthesis. Moreover, we observed modifications in the expression of two transcription factors, C/EBPβ (increased) and Isl-1 (decreased) (Table 1, item 9.0), which may contribute to decreased insulin mRNA expression. Thus, upregulation of C/EBPβ after β-cell exposure to high glucose was shown to inhibit insulin promoter activity (45), whereas downregulation of Isl-1 (Table 1, Fig. 1), a transcription factor involved in β-cell development and possibly in insulin gene transcription (46), may have a negative effect on insulin mRNA expression.

Cytokine-induced inhibition of insulin release in rat

islets is related to decreased glucose oxidation and ATP production (44), but decreased insulin secretion in mouse and human islets is dissociated from inhibition of glucose metabolism (22,47). This suggests that part of the inhibitory effects of cytokines is affected at two distal steps of the insulin release process: ionic fluxes (48) and granule exocytosis (49). In line with this possibility, there were modifications in the expression of several genes encoding for ionic channels (Table 1, item 3.0). Moreover, we detected inhibitory effects of cytokines on the expression of mRNA for soluble NFS attachment protein (SNAP)-25 (mean decrease of –2.9 by IL-1β + INF-γ for 6 h, data not shown), vesicle-associated membrane protein (VAMP)-2, and rab3A (Table 1, item 2.0). These proteins are potential regulators of trafficking, docking, and fusion of secretory vesicles (50). Morphological and biochemical studies demonstrated the presence of VAMP-2 in insulin and in γ-aminobutiric acid (GABA) secretory vesicles of β-cells (51), and cleavage of both VAMP-2 and SNAP-25 by tetanus or botulinum neurotoxins block insulin exocytosis in β-cells (52,53). The rab small *G* protein family consists of nearly 30 members implicated in intracellular vesicle trafficking (54). Rab 3A is associated with the membrane of secretory granules of rat pancreatic β-cells, and overexpression of Rab 3A mutants decreases nutrient-stimulated insulin secretion (55). The expression of the rab-3 GDP/GTP exchange protein, which stimulates the conversion of the inactive form of Rab-3A into the active form (56), is also severely downregulated in cells treated with IL-1β or IL-1β + INF-γ for 24 h (Table 1, item 2.0). This may further aggravate an eventual decrease in insulin secretion mediated by cytokine-induced Rab-3A expression.

Cytokines downregulated expression of mRNAs encoding receptors for the incretins cholecystokinin (CKK)-A, GIP receptor (confirmed by RT-PCR) (Fig. 1), and GLP-1 receptor (Table 1, item 4.0). The roles of GLP-1, GIP, and CKK-A as in vitro and in vivo potentiators of insulin release, via cAMP generation (GIP and GLP-1) and protein kinase C activation (CKK-A), have been confirmed in experimental models, and inhibition of their respective receptors decrease insulin secretion after food intake (57). On the other hand, the fact that cytokines increase expression of the mRNAs for glucagon receptor and downregulate expression of mRNAs for somatostatin, receptor type 2 (Table 1, item 4.0), may to some extent prevent the intracellular decrease in cAMP putatively caused by the downregulation of incretin receptors. The expression of mRNAs encoding receptors for GH and PRL (both confirmed by RT-PCR) (Fig. 1) were also decreased after cytokine treatment. In vivo and in vitro studies have shown that GH and PRL increase mitotic activity in islet cells and stimulate insulin release (58). During pregnancy, upregulation of both PRL and GH receptors contributes to the compensatory increase in β-cell mass and insulin secretion (59). Thus, the observed cytokine-induced downregulation of receptors for incretins and growth factors may hamper both β-cell function in vivo and decrease the ability of these cells to compensate for the progressive immune-mediated β-cell loss.

Several chemokines, cytokines, and cell adhesion molecules were induced by either IL-1β alone or IL-1β + INF-γ (Table 1, items 5.0 and 8.0). Chemokines and cytokines were already highly induced after a 6-h exposure to IL-1β + INF-γ and, with two exceptions, their expression was maintained after a 24-h exposure to IL-1β + INF-γ. IL-1β alone induced expression of four cytokines and chemokines (mob-1 [human IP-10 confirmed by RT-PCR] [Fig. 1], CINC-1, MCP-1, and MIP-3α [confirmed by RT-PCR] [Fig. 1]), while the addition of INF-γ both potentiated the effects of IL-1β and induced expression of five additional chemokines or cytokines, namely fractalkine, osteoprotegerin (both confirmed by RT-PCR) (Fig. 1), macrophage inhibiting cytokine-1, IL-15, and IL-6. The IL-1β–induced expression of CINC-1, CINC-3, and MCP-1 by rat β-cells was previously observed by differential display with RT-PCR (16). MCP-1 attracts mononuclear cells, and recent data indicate that IL-1β also induces MCP-1 mRNA and protein expression in human islets, and that the chemokine is present in pancreatic islets of prediabetic NOD mice (M.-C. Chen, P. Proost, C. Gysemans, C. Mathieu, and D.L.E., manuscript submitted for publication). Besides these previously described chemokines, new cytokine-induced chemokines were observed, including mob-1, fractalkine, and MIP-3α. Mob-1 is a specific chemoattractant for T-helper 1 cells (60) and contributes to autoimmune diseases such as systemic lupus erythematosus (60), autoimmune encephalomyelitis (61), and autoimmune neuritis (62). Fractalkine has adhesive and chemoattractant properties for IL-2–activated NK-cells and $CD8^+$ T-cells (63), whereas MIP-3α has a role in the migration of dendritic cells (64) and was shown to induce adhesion of memory T-cells to ICAM-1 (65) (note that ICAM-1 is also highly induced by cytokines) (Table 1, item 8.0). Among the cytokines expressed in β-cells, IL-15 and IL-6 are of special interest. IL-15 is a potent growth factor for T-, B- and NK-cells, a T-cell chemoattractant, an enhancer of the cytolitic function of effector T- and NK-cells, and a potent inducer of INF-γ production by NK-cells (66,67). The expression of this cytokine was confirmed by RT-PCR (Fig. 1). The expression of IL-6 by β-cells in response to INF-γ and TNF-α has been previously demonstrated (68), and transgenic mice overexpressing IL-6 in the β-cells develop insulitis (69). These results suggest that β-cells exposed to IL-1β and/or INF-γ express several chemokines, cytokines, and adhesion molecules that may potentially contribute to the homing, adhesion, and activation of mononuclear cells in the course of insulitis.

Cytokines also induced several genes related to antigen presentation in β-cells. This was mostly an effect of INF-γ, because IL-1β alone induced only two of these genes, whereas a combination of IL-1β + INF-γ induced 14 genes (Table 1, item 7.0). Previous studies also indicated that INF-γ is the main inducer of MHC class I mRNA and protein in rat and human islet cells (70). We presently observed that IL-1β + INF-γ upregulated several components of the "machinery" for MHC class I antigen presentation (71), including mRNAs for several MHC class I–related components, proteasome subunits, and both MTP-1 and MTP-2 (Table 1, item 7.0). MTP-1 and -2 genes encode proteins that transport peptides (released from the proteasome) from the cytosol to the endoplasmatic reticulum, where they are loaded for MHC class I presentation (71).

It was previously demonstrated that IL-1β induces activation of extracellular signal–related kinase (ERK)-1/2 and

p38 mitogen-activated protein kinase (MAPK) in whole islets (72) and in purified β-cells (13). We presently observed that cytokines upregulate both p38 (mean increase 2.9 by IL-1β + INF-γ for 24 h; data not shown) and ERK-3 (Table 1, item 6.0). There were also modifications in the expression of several of mRNAs encoding enzymes, which may affect signal transduction by MAPKs. Thus, there was a decrease in the expression of MAPK-2 and MAPK phosphatase and an increase in CL100 protein tyrosine phosphatase (Table 1, item 6.0).

Cytokines lead to up- and downregulation of several transcription factors and associated proteins. Among them, the expression of the NF-κB inhibitor I-κBα was highly upregulated after a 6-h exposure by IL-1β + INF-γ (Table 1, item 9.0). After 24 h, the level of expression of this mRNA was still clearly above control levels. IL-1β alone also upregulated I-κBα but to a lesser extent. In parallel, the NF-κB–p105 gene, a precursor of both NF-κB–p50 subunit and the repressor I-κBγ (73), was upregulated at both 6 and 24 h (confirmed by RT-PCR) (Fig. 1 and Table 1, item 9.0). The transcription factor NF-κB is formed by homodimers or heterodimers of Rel/NF-κB proteins, most commonly p50/p65 (74). In nonstimulated cells, NF-κB is sequestered in the cytoplasm associated with the inhibitory molecule I-κB (74). β-cell exposure to IL-1β leads to degradation of I-κBα and nuclear translocation of NF-κB (15). Interestingly, both NF-κB–p105 and IκBα genes are responsive to NF-κB stimulation, which may explain the presently observed increase in the expression of these genes after cytokine exposure (75). The increased expression of I-κBα mRNA and consequent translation of the protein probably functions as a negative feedback for NF-κB activation. Indeed, newly synthesized I-κBα can enter the nucleus, remove NF-κB from the DNA, export the complex back to the cytoplasm, and thus restore the original latent state (74). This negative feedback may explain the decrease in iNOS expression after 24 h in spite of the continuous presence of stimulating cytokines (Fig. 1). Besides I-κBα and NF-κB–p105, we observed expression of 19 additional cytokine-modified genes that are putative target genes for NF-κB. These genes are: AS (increased), iNOS (increased), c-*myc* (increased), mtp-1 (increased), ICAM-1 (increased), MHC class I (increased), IL-15 (increased), IL-6 (increased), mob-1 (increased), MCP-1 (increased), CINC-1/3 (increased), IRF-1 (increased), COX-2 (increased), Osteoprotegerin (increased), MnSOD (increased), HO-1 (increased), and cyclins D1 and D3 (decreased) (41,75). This places NF-κB as a central transcription factor in the process of cytokine-induced β-cell gene expression. In line with this, recent data from our group suggest that blocking NF-κB with an I-κB superrepressor prevents cytokine-induced β-cell apoptosis (H. Heimberg, Y. Heremans, C. Jobin, R.L., M. Darville, D.L.E., manuscript submitted for publication). The transcription factors C/EBPβ and C/EBPδ were also upregulated by cytokines (Table 1, item 9.0) The microarray results for C/EBδ were confirmed by RT-PCR (Fig. 1). C/EBPβ and δ can interact with NF-κB, and both are involved in cytokine-induced MnSOD and Fas expression in pancreatic β-cells (76) (M. Darville and D.L.E., unpublished observations).

The induction of putative β-cell defense/repair genes was more evident after 24-h exposure to IL-1β + INF-γ than after exposure to IL-1β alone (Table 1, item 12.0). This is in line with previous observations suggesting that a combination of IL-1β + INF-γ induces more severe β-cell damage than IL-1β alone (4,5). Besides previously described cytokine-induced genes, such as hsp-70 and MnSOD (17), we observed induction of hsp-27, hsp-40, GADD-153 (confirmed by RT-PCR) (Fig. 1), *O*-6 methylguanine-DNA methyltransferase (MGMT), gas-5 growth arrest homolog, and methallothionein and MX1 gene (Table 1, item 12.0). Several of these genes are involved in DNA repair and are probably a β-cell response to nitric oxide–induced DNA damage (5). Upregulation of these genes may explain the protective effects of a short-term (24 h) β-cell exposure to low concentrations of IL-1β against a subsequent assault by alloxan, streptozotocin, or NO (37). However, this increased expression of defense/repair genes is not sufficient to prevent β-cell apoptosis after a prolonged (6–9 days) exposure to IL-1β + INF-γ. This may, at least in part, be caused by the fact that cytokines also downregulate important defense/repair genes, such as gas-6 growth arrest (a ligand of receptor tyrosine kinases AXL, Sky, and Mer that have protective effects against apoptosis caused by serum deprivation, myc overexpression, and TNF-α in NIH3T3 cells) (77) and glutathione peroxidase (an antioxidant enzyme) (Table 1, item 12.0).

Only one gene directly related to apoptosis regulation was found modified more than threefold by cytokines in the microarray analysis. Thus, the death protein-5 was increased at 24 h by both exposure to IL-1β alone and IL-1β + INF-γ. Death protein-5 is a gene induced in neuronal apoptosis, and it possesses a BH3 domain that allows interaction with Bcl-2 and Bcl-xl (78). It will be of interest to investigate whether this gene has a similar role in β-cells. The lack of observed cytokine-induced changes in several of the known pro- and antiapoptotic transcripts present in the Affymetrix array (such as Bcl-2, Bcl-x, Bad, and caspases) raises concern whether the time points selected for the present analysis (6 and 24 h) were the most adequate to detect modifications in these genes. Cytokine-induced β-cell death increases mostly after 3–6 days (10–14), and we cannot exclude that an array analysis performed after 36 or 48 h of cytokine exposure would detect modifications in the classic pro- and antiapoptotic genes. An alternative possibility is that β-cell death is not determined by changes in the known pro- and antiapoptotic genes but instead depends on modifications in the expression of several genes related to maintenance of β-cell phenotype and function. Additional experiments are required to clarify this issue.

A model of the effects of cytokines in pancreatic β-cells, based on the present array analysis, is provided in Fig. 2. Cytokines decrease the expression of several genes related to differentiated β-cell function and preservation of β-cell mass, including insulin, GLUT2, glucokinase, and diverse receptors for incretins and growth hormones. This loss of β-cell–specialized functions is probably associated to cytokine-induced decrease in the expression of Isl-1 (present data) and Pdx-1 (37) and may explain the impaired glucose-induced insulin release observed in prediabetic NOD mice (79). On the other hand, there is upregulation of stress-response genes and, surprisingly for

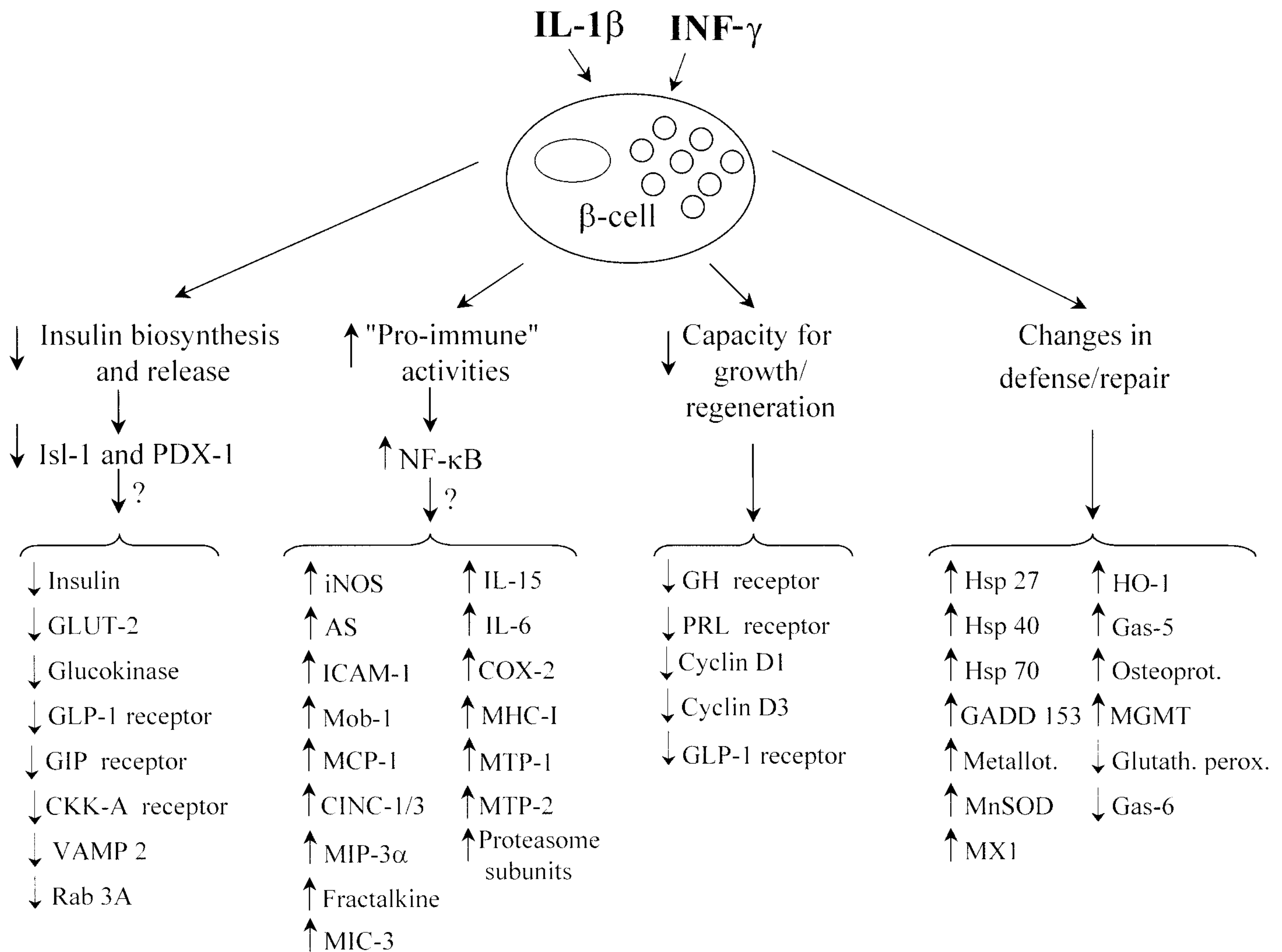

FIG. 2. Proposed model based on the present microarray findings of the role for cytokines in the process of β-cell dysfunction and death in early type 1 diabetes. It is conceivable that a decrease in Isl-1 and PDX-1 expression and an increase in NF-κB activity contribute, respectively, to modifications in mRNAs related to insulin biosynthesis and release or proimmune activities.

such a differentiated cell, expression of genes encoding several chemokines, cytokines, and adhesion molecules, several having the potential to increase mononuclear cell homing and activity during insulitis. Most of these genes are probably upregulated as a consequence of NF-κB activation. Note that NF-κB–responsive genes may also directly contribute to β-cell apoptosis (H. Heimberg, Y. Heremans, S. Jobin, R.L., A.K.C., M. Darville, D.L.E., manuscript submitted for publication).

The present findings open new avenues for research on the mechanisms of immune-mediated β-cell death, and we intend to characterize in detail the function of several of the novel β-cell genes, both at the mRNA and protein levels. The picture that emerges from the present data is that β-cells are not passive bystanders of their own destruction. They respond to immune-mediated damage by triggering complex patterns of gene expression, with some of these genes aggravating β-cell damage, whereas others probably contribute to cell defense/repair (Fig. 2). At some point in this response, the balance is tilted toward β-cell death. A more detailed characterization of the gene patterns described in the present study and of the transcription factors regulating them may allow us to understand what tilts the balance in this direction. Hopefully, this knowledge will point to novel targeted approaches to improve β-cell survival in early type 1 diabetes.

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