Nuclear Factor-κB Regulates β-Cell Death
A Critical Role for A20 in β-Cell Protection

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Apoptotic β-cell death is central to the pathogenesis of type 1 diabetes and may be important in islet graft rejection. Despite this, genetic control of β-cell apoptosis is only poorly understood. We report that inhibition of gene transcription sensitized β-cells to tumor necrosis factor (TNF)-α–induced apoptosis, indicating the presence of a regulated antiapoptotic response. Using oligonucleotide microarrays and real-time PCR, we identified TNFAIP3/A20 as the most highly regulated antiapoptotic gene expressed in cytokine-stimulated human and mouse islets. Cytokine induction of A20 mRNA in primary islets and insulinoma cells was rapid and observed within 1 h, consistent with A20 being an immediate early response gene in β-cells. Regulation of A20 was nuclear factor-κB (NF-κB)–dependent, two NF-κB sites within the A20 promoter were found to be necessary and sufficient for A20 expression in β-cells. Activation of NF-κB by TNF receptor–associated factor (TRAF) 2, TRAF6, NF-κB–inducing kinase, or protein kinase D, which transduce signals downstream of Toll-like receptors, TNF receptors, and free radicals, respectively, were all potent activators of the A20 promoter. Moreover, A20 expression was induced in transplanted islets in vivo. Finally, A20 expression was sufficient to protect β-cells from TNF-induced apoptosis. These data demonstrate that A20 is the cardinal antiapoptotic gene in β-cells. Further, A20 expression is NF-κB dependent, thus linking islet proinflammatory gene responses with protection from apoptosis.
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Apoptosis or programmed cell death is a genetically controlled response of the cell to commit suicide (1,2). Apoptosis is the physiological process for cell deletion in normal, reorganizing, or involuting tissue and is required for shaping of the endocrine pancreas (3,4). Aside from its role in normal cell biology, β-cell apoptosis has been implicated in the pathophysiology of type 1 diabetes, both at its initiation phase and as the final effector mechanism (5,6). Evidence from the NOD mouse (a widely studied model of autoimmune diabetes) (7) indicates that autoreactive cytolytic T-cells (8), as well as soluble mediators including proinflammatory cytokines and free radicals, contribute to increased β-cell apoptotic destruction during the pathogenesis of type 1 diabetes (6,9). Transplantation of islets is considered to be one potential approach that could restore normal metabolic control for the cure of type 1 diabetes. However, multiple obstacles are faced in islet transplants, including cellular rejection akin to the mechanisms involved in autoimmune destruction of β-cells, and also primary nonfunction, a phenomena related to lack of nutrients, hypoxia, and nonspecific inflammatory mediators (10–12).

Despite the importance of apoptosis in the pathophysiology of β-cell death, the genetic control of apoptosis in islets is poorly understood. Determining the molecular basis of β-cell susceptibility to apoptosis would increase our understanding of the mechanisms underscoring β-cell loss, as well as reveal potential gene therapy candidates for the creation of "death-defying" islets (13), capable of resisting immune and nonimmune insults. With this goal in mind, we generated a custom microarray, a so-called "death-CHIP," to map the immediate early antiapoptotic gene expression profile of cytokine-activated islets. Though later changes in islet gene expression after cytokine activation have been mapped in a number of studies (14,15), we focused only on the immediate early response, as it is this response that determines cell fate after inflammatory insult (16–19). Surprisingly, we found that in contrast to other cell types, primary islets have a highly restricted immediate early antiapoptotic gene response, with TNFAIP3/A20 being the most highly regulated antiapoptotic gene. Significantly, we demonstrate that A20 is regulated at the level of gene transcription in pancreatic β-cells by the proinflammatory transcription factor nuclear factor-κB (NF-κB), thus linking islet inflammatory gene responses with protection from apoptosis. Together, with our previous studies demonstrating an anti-inflammatory and antiapoptotic function for A20 (13,20–22), these present data indicate that A20 is a critical component of the islet-regulated response to inflammatory stress and injury. Thus, we demonstrate that loss of A20 expression renders β-cells susceptible to apoptotic death; conversely, enhancing A20 expression in β-cells may improve their survival in the face of inflammatory and autoimmune insults (13,21).

RESEARCH DESIGN AND METHODS

BALB/c and C57BL/6 mice were purchased from ARC (Perth, WA, Australia). NOD mice were purchased from The Walter and Eliza Hall Institute (Melbourne, VIC, Australia). Procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

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FACS, fluorescence-activated cell sorter; FADD, Fas-associated death domain; FADD-DN, FADD dominant negative inhibitor; β-gal, β-galactosidase; IL, interleukin; IFN, interferon; IκBα, inhibitor of NF-κB; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; PKD, protein kinase D; Th, T-helper; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNF-R, TNF receptor; TRAF, TNF receptor–associated factor.

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Cell death assays. Assays were performed using a modified crystal violet method (23,24). Briefly, β-insulinoma (β-TC_3) cells were pretreated (30 min) with 0.01 μmol/l actinomycin D (Calbiochem, Kilsyth, Australia) before the addition of 100, 500, or 1000 units/ml of recombinant mouse tumor necrosis factor (TNF)-α (R&D Systems, Minneapolis, MN). Twenty-four hours later, cell death was determined as described (23).

Cytokine stimulation. Human primary islets, provided by Prof. Phillip O'Connell (Westmead Hospital, Sydney, NSW, Australia), were isolated from cadaveric organ donors and cultured as described (25). Mouse islets were isolated and cultured as we have described (13,26). For cytokine stimulation, islets (150–200 islets/treatment) were stimulated with 200 units/ml of the appropriate cytokine for the indicated times. Human and mouse recombinant T-helper (Th) 1 cytokines interleukin (IL)-1β, TNF-α, or γ-interferon (IFN), and the Th2 cytokines IL-4, IL-13, or IL-15 (R&D Systems). β-TC_3 and Min6 cells were cultured as described (27). Cells were stimulated with 200 units/ml of mouse recombinant IL-1β or TNF-α for the indicated time points. In some cases islets were first pretreated with 100 μmol/l of the pharmacological NF-κB inhibitor, pyrrolidine dithiocarbamate (Sigma, St. Louis, MO) as described (28).

Gene expression in β-cells sorted with a fluorescence-activated cell sorter. For cell sorting, primary mouse islets were first stimulated with cytokines as above and then washed (4% EDTA-PBS), and dispersed into single cells by a brief incubation with 0.05% trypsin-EDTA (Invitrogen). Dispersed islets were then washed free of trypsin and resuspended in 1% BSA-PBS and sorted in a fluorescence-activated cell sorter (FACS) based upon autofluorescence (FL-1) as described (29). Sorted cells were then analyzed for A20 expression as described below. The percentage of insulin-positive β-cells in the $FL\text{-}1^{Hi}$ and $FL\text{-}1^{Lo}$ sorted populations was determined by insulin (mouse anti-insulin; Zymed Laboratories, South San Francisco, CA) and glucagon (rabbit anti-glucagon; ICN Immunologicals, Lisle, IL) specific labeling on cytospun cells.

Real-time PCR. mRNA was extracted as described (26); we obtained ~3–5 μg mRNA per 200 islets. cDNA was produced from 0.5 μg of RNA using the SuperScript III Reverse Transcriptase Kit (Invitrogen, Australia). Reactions were performed on the RG300 Real Time PCR System (Rotorgene), using SYBR green chemistry (JumpStart, Sigma, Castle Hill, Australia). A20 primers were human (F: AAAGCCCTCATCGACAGAAA; R: CAGTTGCCAGCGGAATTTA) and mouse (F: TGGTTCCAATTTTGCTCCTT; R: CGTTGATCAGGTGAGTCGTG). Human and mouse samples were normalized with glyceraldehyde-3-phosphate dehydrogenase (F: CACATCAAGAAGGTGGTG; R: TGTCATACCAGGAAATGA) or CPH2, respectively (F: TGGACCAAACACAAACGGTTCC; R: ACATTGCGAGCAGATGGGGTAG). Fold change was calculated following the $2\Delta\Delta^{CT}$ method.

Microarray. Before hybridization, total RNA was amplified and labeled using the MessageAmp II aRNA Amplification Kit (Ambion, Austin, TX). Amplified RNA quality was evaluated by capillary electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Samples were hybridized on a custom-designed death-CHIP, a CustomArray 12K-Microarray platform (CombiMatrix, Mukilteo, WA). Genes were selected for inclusion on the microarray based upon their reported role in the regulation of cell death (Table 1). The microarray probe design and feature layout, hybridization and scanning protocol, data normalization, and quality control data will be reported elsewhere (D. Liuwantara, S.T. Grey, unpublished data). All data were first normalized and background corrected, then log (base 2) transformed. For each experiment, islets were isolated from 10 mice, pooled, and treated with cytokines, and RNA was harvested as described. Each experiment was performed on three (NOD) or four (BALB/c) independent islet preparations. All samples were run on duplicate arrays; 42 arrays were run in total. The mean coefficients of variation (CVs) based on replicate features on the microarrays were ≤20%.

Transfection and reporter assay. Transfection of β-TC_3 cells, luciferase, and β-galactosidase (β-gal) activity was performed as described (21). The A20 reporter constructs were a kind gift of Dr. R. Dikstein (The Weizmann Institute of Science, Rehovot, Israel) (30). The expression plasmids encoding Fas-associated death domain (FADD), TNF receptor–associated factor (TRAF) 2, TRAF6, NF-κB–inducing kinase (NIK), P65, and protein kinase D (PKD) have been described (22,31,32). All data are expressed as relative light activity (21).

Islet transplantation. Islet transplantation into diabetic (blood glucose ≥300 mg/dl) recipients was performed essentially as described (13). Islets (isolated from three donor mice per recipient) from BALB/c mice were transplanted either into diabetic BALB/c recipients (syngeneic model) or into diabetic C57BL/6 mice (allogeneic model). To harvest the transplanted islets, nephrectomy was performed on postoperative day 5. The islet graft was collected by carefully peeling off the kidney capsule; mRNA was then isolated as described above. In some cases the graft was prepared for histological assessment as described (13).

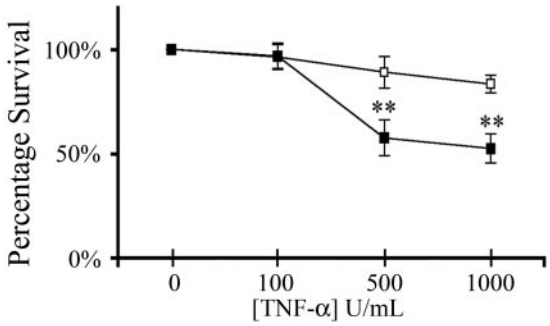

FIG. 1. β-Cells have an inducible antiapoptotic response. Survival of β-TC_3 cells treated with increasing concentrations of TNF-α with (■) or without (□) Actinomycin D. Data represent mean ± SD percentage survival from four independent experiments, performed in duplicate. Data with 500 and 1,000 units are significant ($P \leq 0.005$).**

RESULTS

β-Cells have an inducible antiapoptotic response. Despite the importance of cell death in pathological β-cell loss, little is known about the genetic control of apoptosis in these cells. Cell death is a highly regulated event, controlled in a proinflammatory setting by an inducible set of antiapoptotic genes rapidly upregulated in response to this same inflammatory stress and/or injury (16–19). We were particularly interested in determining whether β-cells could also generate a rapid and inducible antiapoptotic response after exposure to inflammatory cytokines as has been described for other cell types (16–18). We first tested this concept by stimulating β-TC_3 cells with TNF-α in the presence or absence of the general transcription inhibitor, actinomycin D. Blockade of de novo gene transcription in combination with TNF-α stimulation sensitized β-TC_3 cells to cell death, whereas either treatment alone had minimal effect upon cell survival (Fig. 1). Cell survival decreased from 100% in control cells to 53% when cells were treated with 1000 units of TNF-α and actinomycin D ($P \leq 0.005$). These data demonstrate that blockade of de novo gene transcription sensitizes β-cells to TNF-α–induced cell death, consistent with the notion that TNF-α triggers a proapoptotic death cascade that can be prevented by gene induction (16–18). Thus, β-cells do have a cytokine-dependent regulated antiapoptotic response as has been demonstrated for other cell types.

Islets exhibit a limited immediate early antiapoptotic gene response. Having established that β-cells do have an inducible protective response, we next determined the molecular basis of this response. We used a custom-designed microarray (death-CHIP) to analyze the immediate early antiapoptotic gene response of primary rodent islets. Antiapoptotic genes from all known and putative antiapoptotic gene families were included on the death-CHIP (Table 1). cRNA was prepared from islets isolated from nondiabetic-prone BALB/c mice or diabetic-prone NOD mice. For each islet preparation, islets were either left untreated or stimulated with IL-1β or TNF-α for 1 h. These cytokines were chosen based on their established roles in inducing β-cell apoptosis both in vitro (21,33,34) and in vivo (35–37).

Of the 63 antiapoptotic genes represented on the death-CHIP, we found that islets constitutively expressed a broad selection of antiapoptotic genes over a range of expression levels (Table 1). To confirm the findings of the death-CHIP, the basal expression of selected genes (Table 1) was established by Western blot and/or PCR analysis in

TABLE 1
Analysis of inducible antiapoptotic genes expressed in islets from BALB/c or NOD mice

Gene symbol	Gene title	GenBank ID	Basal expression	Fold Δ IL-1/media	*P* value	Fold Δ TNF/media	*P* value
BALB/c							
Aatf	Apoptosis antagonizing transcription factor	NM_019816	1,356.2	0.792	0.076	0.774	0.099
Aven	Apoptosis caspase activation inhibitor	NM_028844	173.1	1.004	0.988	1.100	0.758
Birc1a	Baculoviral IAP repeat-containing 1a	NM_008670	492.9	0.873	0.612	0.916	0.771
Birc1b	Baculoviral IAP repeat-containing 1b	NM_010872	1,094.9	0.836	0.242	0.854	0.170
Birc1e	Baculoviral IAP repeat-containing 1e	NM_010870	358.3	0.804	0.387	0.871	0.579
Birc1f	Baculoviral IAP repeat-containing 1f	NM_010871	904.1	0.963	0.842	1.038	0.839
Birc2	Baculoviral IAP repeat-containing 2*	NM_007465	1,069.4	1.045	0.635	1.166	0.122
Birc3	Baculoviral IAP repeat-containing 3*	NM_007464	422.2	2.86	0.00004550	2.252	0.00030850
Birc4	Baculoviral IAP repeat-containing 4*	NM_009688	490.2	0.851	0.497	0.810	0.375
Birc5	Baculoviral IAP repeat-containing 5	NM_009689	1,462.4	1.076	0.710	1.067	0.757
Birc6	Baculoviral IAP repeat-containing 6	NM_007566	715.6	1.086	0.755	1.205	0.593
Bcl2	B-cell leukemia/lymphoma 2*	NM_009741	145.5	0.854	0.358	0.872	0.312
Bcl2a1a	B-cell leukemia/lymphoma 2 related protein A1a*	NM_009742	297.4	1.259	0.230	1.171	0.405
Bcl2a1c	B-cell leukemia/lymphoma 2 related protein A1c	NM_007535	421.3	1.5	0.061	1.517	0.058
Bcl2a1d	B-cell leukemia/lymphoma 2 related protein A1d	NM_007536	269.3	1.201	0.385	1.112	0.597
Bcl3	B-cell leukemia/lymphoma 3	NM_033601	1,067.2	1.249	0.137	1.441	0.042
Bag4	BCL2-associated athanogene 4	NM_026121	242.7	0.681	0.017	0.883	0.425
Bcl2l	Bcl2-like*	NM_009743	347.5	0.888	0.500	0.917	0.454
Bcl2l10	Bcl2-like 10	NM_013479	1,548.8	0.883	0.514	0.975	0.865
Bcl2l2	Bcl2-like 2	NM_007537	1,140.7	0.827	0.215	0.903	0.632
Bfar	Bifunctional apoptosis regulator	NM_025976	1,554.3	0.946	0.747	0.965	0.811
Dad1	Defender against cell death 1	NM_010015	8,589.7	0.814	0.073	0.889	0.454
Gpx1	Glutathione peroxidase 1	NM_008160	3,801.6	1.297	0.334	1.431	0.237
Gpx2	Glutathione peroxidase 2	NM_030677	2,112.7	0.962	0.794	0.850	0.198
Gpx4	Glutathione peroxidase 4	NM_008162	4,202.2	1.022	0.915	1.087	0.735
Gpx5	Glutathione peroxidase 5	NM_010343	3,446.1	0.989	0.924	0.939	0.702
Hras1	Harvey rat sarcoma virus oncogene 1	NM_008284	2,061	0.861	0.155	0.920	0.418
Hspa5	Heat shock 70 kD protein 5 (glucose-regulated protein)*	NM_022310	10,768.6	1.07	0.789	1.183	0.586
Hspe1	Heat shock protein 1 (chaperonin 10)	NM_008303	3,781.1	0.985	0.950	1.195	0.537
Hspd1	Heat shock protein 1 (chaperonin)	NM_010477	3,407.4	0.975	0.860	1.127	0.449
Hspca	Heat shock protein 1 α	NM_010480	8,052.7	1.102	0.706	1.357	0.268
Hspcb	Heat shock protein 1 β	NM_008302	12,568.9	0.887	0.429	1.144	0.416
Hsp105	Heat shock protein 105	NM_013559	2,763.3	1.005	0.985	1.499	0.147
Hspa12b	Heat shock protein 12B	NM_028306	360.4	1.023	0.897	0.976	0.896
Hspa1a	Heat shock protein 1A	AW763765	228.4	1.338	0.663	1.942	0.271
Hspa1b	Heat shock protein 1B	M12573	3,598.2	1.139	0.741	1.506	0.238
Hspa1l	Heat shock protein 1-like	NM_013558	437.9	0.835	0.286	0.806	0.235
Hspa2	Heat shock protein 2	NM_008301	327.2	1.028	0.897	0.986	0.958
Hspb3	Heat shock protein 3	NM_019960	516.5	0.97	0.894	0.870	0.408
Hspa4	Heat shock protein 4	NM_008300	2,515.1	1.005	0.978	1.161	0.437
Hspa8	Heat shock protein 8	NM_031165	9,024.8	1.233	0.416	1.456	0.199
Hspb7	Heat shock protein family member 7 (cardiovascular)	NM_013868	1,878.8	0.656	0.169	0.713	0.217
Hebp2	Heme binding protein 2	NM_019487	2,216.5	0.784	0.068	0.710	0.052
Hmox1	Heme oxygenase (decycling) 1*	NM_010442	5,708.6	0.904	0.317	0.891	0.234
Hmox2	Heme oxygenase (decycling) 2	NM_010443	1,657.5	0.939	0.682	1.034	0.866
Hnf4	Hepatic nuclear factor 4	NM_008261	895.2	0.933	0.581	1.037	0.753
Sod1	Superoxide dismutase 1 soluble*	BM240246	228.6	1.132	0.690	1.536	0.169
Sod1	Superoxide dismutase 1 soluble	BC002066	15,483.3	0.942	0.712	0.971	0.911
Sod2	Superoxide dismutase 2 mitochondrial*	NM_013671	4,534.3	1.305	0.175	1.195	0.461
Sod3	Superoxide dismutase 3 extracellular	NM_011435	5,812.8	0.729	0.147	0.616	0.088
Txn1	Thioredoxin 1	NM_011660	5,806	0.925	0.544	1.064	0.623
Txn2	Thioredoxin 2	NM_019913	1,676	0.901	0.208	0.999	0.992
Txndc1	Thioredoxin domain containing 1	NM_028339	2,152.5	0.878	0.401	1.000	0.999
Txndc5	Thioredoxin domain containing 5	NM_145367	2,078.1	0.971	0.836	1.017	0.924
Txnip	Thioredoxin interacting protein	NM_023719	1,257.5	1.293	0.397	1.513	0.240
Txnrd1	Thioredoxin reductase 1	NM_015762	2,784.4	0.939	0.714	0.963	0.851

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TABLE 1
Continued

Gene symbol	Gene title	GenBank ID	Basal expression	Fold Δ IL-1/media	*P* value	Fold Δ TNF/media	*P* value
Txnrd2	Thioredoxin reductase 2	NM_013711	633.6	0.827	0.416	0.858	0.472
Txnrd3	Thioredoxin reductase 3	NM_153162	148.4	0.988	0.944	1.106	0.571
Txnl	Thioredoxin-like	NM_016792	304	0.765	0.071	0.813	0.051
Txnl2	Thioredoxin-like 2	NM_023140	2,667.9	1.001	0.996	1.161	0.483
Tnfaip6	Tumor necrosis factor α–induced protein 6	NM_009398	121.4	1.305	0.433	1.179	0.632
Tnfaip1	Tumor necrosis factor α–induced protein 1	NM_009395	3,090.2	0.934	0.568	1.058	0.534
Tnfaip2	Tumor necrosis factor α–induced protein 2	NM_009396	590	1.14	0.416	0.905	0.590
Tnfaip3	Tumor necrosis factor α–induced protein 3*	NM_009397	117.6	10.612	0.0000052	5.917	0.00014460
NOD							
Aatf	Apoptosis antagonizing transcription factor	NM_019816	1,130.10	0.842	0.199	0.985	0.914
Aven	Apoptosis caspase activation inhibitor	NM_028844	145.30	1.364	0.431	1.304	0.493
Birc1a	Baculoviral IAP repeat-containing 1a	NM_008670	375.80	0.643	0.365	0.789	0.628
Birc1b	Baculoviral IAP repeat-containing 1b	NM_010872	1,020.50	0.813	0.437	0.875	0.593
Birc1e	Baculoviral IAP repeat-containing 1e	NM_010870	314.00	0.696	0.075	0.936	0.678
Birc1f	Baculoviral IAP repeat-containing 1f	NM_010871	933.10	0.666	0.094	0.841	0.350
Birc2	Baculoviral IAP repeat-containing 2*	NM_007465	1,067.40	1.263	0.024	1.255	0.004
Birc3	Baculoviral IAP repeat-containing 3*	NM_007464	429.90	3.742	0.013	2.695	0.039
Birc4	Baculoviral IAP repeat-containing 4*	NM_009688	350.20	0.692	0.103	0.795	0.201
Birc5	Baculoviral IAP repeat-containing 5	NM_009689	1,555.30	0.736	0.176	0.987	0.947
Birc6	Baculoviral IAP repeat-containing 6	NM_007566	945.70	1.427	0.381	1.344	0.447
Bcl2	B-cell leukemia/lymphoma 2*	NM_009741	183.00	0.889	0.304	1.052	0.647
Bcl2a1a	B-cell leukemia/lymphoma 2–related protein A1a*	NM_009742	519.60	1.639	0.488	1.323	0.720
Bcl2a1c	B-cell leukemia/lymphoma 2–related protein A1c	NM_007535	689.80	1.733	0.172	1.416	0.436
Bcl2a1d	B-cell leukemia/lymphoma 2–related protein A1d	NM_007536	450.60	1.703	0.495	1.340	0.731
Bcl3	B-cell leukemia/lymphoma 3	NM_033601	933.90	1.548	0.152	1.479	0.215
Bag4	BCL2-associated athanogene 4	NM_026121	238.50	0.844	0.187	0.992	0.931
Bcl2l	Bcl2-like*	NM_009743	334.90	0.951	0.788	0.901	0.606
Bcl2l10	Bcl2-like 10	NM_013479	1,782.80	0.624	0.139	0.777	0.408
Bcl2l2	Bcl2-like 2	NM_007537	1,340.40	0.938	0.741	1.038	0.838
Bfar	Bifunctional apoptosis regulator	NM_025976	1,541.90	0.708	0.076	0.844	0.304
Dad1	Defender against cell death 1	NM_010015	12,015.20	0.947	0.417	0.993	0.926
Gpx1	Glutathione peroxidase 1	NM_008160	2,953.10	1.586	0.066	1.631	0.053
Gpx2	Glutathione peroxidase 2	NM_030677	2,011.40	0.702	0.076	0.829	0.191
Gpx4	Glutathione peroxidase 4	NM_008162	4,327.80	1.171	0.657	1.295	0.477
Gpx5	Glutathione peroxidase 5	NM_010343	4,482.00	0.573	0.042	0.799	0.314
Hras1	Harvey rat sarcoma virus oncogene 1	NM_008284	2,310.70	0.781	0.067	0.912	0.400
Hspa5	Heat shock 70 kD protein 5 (glucose-regulated protein)*	NM_022310	11,382.50	1.443	0.578	1.242	0.737
Hspe1	Heat shock protein 1 (chaperonin 10)	NM_008303	3,376.70	1.395	0.504	1.473	0.441
Hspd1	Heat shock protein 1 (chaperonin)	NM_010477	2,855.00	1.099	0.470	1.241	0.198
Hspca	Heat shock protein 1 α	NM_010480	7,447.90	1.495	0.187	1.377	0.284
Hspcb	Heat shock protein 1 β	NM_008302	11,423.10	1.070	0.454	1.070	0.404
Hsp105	Heat shock protein 105	NM_013559	2,676.50	1.242	0.287	1.155	0.460
Hspa12b	Heat shock protein 12B	NM_028306	370.90	0.817	0.188	0.903	0.470
Hspa1a	Heat shock protein 1A	AW763765	329.20	1.308	0.643	1.172	0.785
Hspa1b	Heat shock protein 1B	M12573	3,062.50	0.938	0.880	1.019	0.957
Hspa1l	Heat shock protein 1-like	NM_013558	442.30	0.680	0.047	0.794	0.075
Hspa2	Heat shock protein 2	NM_008301	355.40	1.391	0.227	1.443	0.170
Hspb3	Heat shock protein 3	NM_019960	466.30	0.652	0.111	0.758	0.131
Hspa4	Heat shock protein 4	NM_008300	2,247.70	1.302	0.315	1.361	0.241
Hspa8	Heat shock protein 8	NM_031165	9,660.00	1.486	0.284	1.479	0.297
Hspb7	Heat shock protein family member 7 (cardiovascular)	NM_013868	1,487.30	0.663	0.461	0.786	0.647
Hebp2	Heme binding protein 2	NM_019487	2,048.90	0.745	0.259	0.818	0.428
Hmox1	Heme oxygenase (decycling) 1*	NM_010442	5,759.00	0.799	0.359	0.894	0.609
Hmox2	Heme oxygenase (decycling) 2	NM_010443	1,226.50	1.174	0.486	1.267	0.304
Hnf4	Hepatic nuclear factor 4	NM_008261	687.50	1.146	0.611	1.220	0.467
Sod1	Superoxide dismutase 1 soluble	BM240246	277.20	0.630	0.104	0.757	0.177
Sod1	Superoxide dismutase 1 soluble	BC002066	19,857.70	1.082	0.319	1.149	0.071

Continued on following page

TABLE 1
Continued

Gene symbol	Gene title	GenBank ID	Basal expression	Fold Δ IL-1/ media	*P* value	Fold Δ TNF/ media	*P* value
Sod2	Superoxide dismutase 2 mitochondrial*	NM_013671	5,852.10	1.200	0.290	1.052	0.802
Sod3	Superoxide dismutase 3 extracellular	NM_011435	4,550.40	0.677	0.011	0.712	0.031
Txn1	Thioredoxin 1	NM_011660	6,255.90	1.102	0.389	1.164	0.113
Txn2	Thioredoxin 2	NM_019913	1,779.30	0.957	0.731	1.015	0.911
Txndc1	Thioredoxin domain containing 1	NM_028339	2,646.10	1.093	0.564	1.134	0.429
Txndc5	Thioredoxin domain containing 5	NM_145367	1,720.00	1.154	0.353	1.267	0.120
Txnip	Thioredoxin interacting protein	NM_023719	928.30	2.014	0.005	1.395	0.156
Txnrd1	Thioredoxin reductase 1	NM_015762	1,685.20	1.550	0.234	1.538	0.239
Txnrd2	Thioredoxin reductase 2	NM_013711	601.00	0.641	0.158	0.764	0.342
Txnrd3	Thioredoxin reductase 3	NM_153162	116.60	1.175	0.258	1.374	0.093
Txnl	Thioredoxin-like	NM_016792	327.60	1.005	0.970	1.019	0.900
Txnl2	Thioredoxin-like 2	NM_023140	2,450.00	1.320	0.438	1.299	0.451
Tnfaip6	Tumor necrosis factor α–induced protein 6	NM_009398	142.00	1.259	0.590	1.151	0.716
Tnfaip1	Tumor necrosis factor α–induced protein 1	NM_009395	3,049.80	1.071	0.625	1.067	0.630
Tnfaip2	Tumor necrosis factor α–induced protein 2	NM_009396	561.50	1.048	0.925	0.928	0.878
Tnfaip3	Tumor necrosis factor α–induced protein 3*	NM_009397	248.70	8.884	0.019	3.610	0.115

*Genes validated by PCR and/or Western blot.

primary human and rodent islets and β-insulinoma cell lines (data not shown). Of the genes confirmed to be expressed in all three tissue samples, highly expressed genes (≥10,000 mean intensity units) including heat shock protein 70, HSPA1A/HSP70, and SOD1/CuZnSOD; moderately expressed genes (1–10,000 mean intensity units) including Heme oxygenase 1 and SOD2/MnSOD; and genes including the cellular inhibitors of apoptosis, BIRC2/c-IAP1, BIRC3/c-IAP2, BIRC4/XIAP, and the BCL family genes BCL2, BCL2L1/BCLXL, BCL2A1A/Bfl-1/A1, and TNFAIP3/A20 were expressed at relatively low constitutive basal levels (≤1,000 mean intensity units). In contrast, examination of the expression profile after cytokine stimulation indicated that islets have a relatively sparse immediate early antiapoptotic gene response (Fig. 2*A* and *B*). Establishing a twofold cutoff for statistical significance, only two genes were determined to be upregulated, namely, TNFAIP3/A20 and BIRC3/c-IAP-2, of which A20 was consistently the most highly regulated antiapoptotic gene (Fig. 2*C*). These data contrast islets with other tissues such as endothelial cells, which express a broad range of antiapoptotic genes under similar conditions (19,38,39).

Regulation of A20 in rodent islets. As A20 was the most highly regulated immediate early antiapoptotic gene in islets, we focused on its regulation in more detail. Primary islets isolated from BALB/c or NOD mice were stimulated with the cytokines IL-1β or TNF-α for 1–8 h, and steady-state mRNA expression was analyzed by PCR. As shown in Fig. 3*A*, A20 was expression was markedly upregulated by ~23-fold ($P \leq 0.005$) and ~16-fold ($P \leq 0.001$) in BALB/c and NOD islets, respectively, within 1 h after IL-1β stimulation. Kinetic analysis of A20 expression revealed that although the expression declined thereafter, it was still maintained at significant levels. Thus, A20 is a cytokine-inducible immediate early response gene in islets, consistent with its regulation in other cell types (40). In comparison with IL-1β, TNF-α was a relatively poor inducer of A20 expression, stimulating only an approximately fourfold increase in A20 mRNA expression at 1 h for islets isolated from either BALB/c or NOD mice ($P \leq 0.05$). This finding may relate to differential activation of NF-κB family members by IL-1β versus TNF-α, respectively, which can then lead to distinct patterns of gene expression (41). The poor induction of A20 by TNF-α is interesting, given the fact that TNF-α is one of the earliest β-cell–toxic cytokines detected in the islet infiltrate during the development of type 1 diabetes in NOD mice (36). However, Fig. 3*B* shows that there was no difference in A20 expression between the male and female NOD mice, demonstrating that the lower disease incidence in the male NOD mice is not associated with A20 expression.

Regulation of A20 in human islets. We next examined A20 regulation in primary human islets. As shown in Fig. 3*C*, A20 mRNA expression was rapidly induced after IL-1β stimulation, reaching a maximal ~25-fold induction at 1 h after stimulation and decreasing thereafter. Again IL-1β was a more potent inducer of A20 expression than TNF-α. In contrast with IL-1β and TNF-α, other Th1-type cytokines such as γ-IFN and IL-15 and the Th2-type cytokines IL-4 and IL-13 did not induce A20 expression in human or rodent islets (data not shown). Together, these data demonstrate that A20 is an immediate early response gene in both human and rodent islets, preferentially regulated by the Th1-type cytokines, IL-1β and TNF-α.

β-Cell–specific expression of A20 in islets. Islets of Langerhans are a heterogeneous tissue comprising not only insulin-secreting β-cells but also additional hormone-secreting cells including α- and γ-cells. β-Cells from freshly isolated islets are autofluorescent due to intracellular flavin adenine dinucleotide levels and can be FACS-purified on this basis (29). To determine the cellular source of A20 expression in intact primary islets, we next examined A20 expression in FACS-purified primary β-cells and non–β-cells (Fig. 4*A*). We found that inducible A20 expression was restricted to insulin-positive β-cells (approximately threefold induction $P \leq 0.05$) as A20 expression was not induced by IL-1β in glucagon-positive cells (Fig. 4*B*). We also examined inducible A20 expression in the insulinoma cell line, Min6. Stimulation of Min6 cells with IL-1β or TNF-α resulted in a rapid and marked induction of A20, with kinetics similar to that seen for primary human and rodent islets (Fig. 4*C*). Thus, these

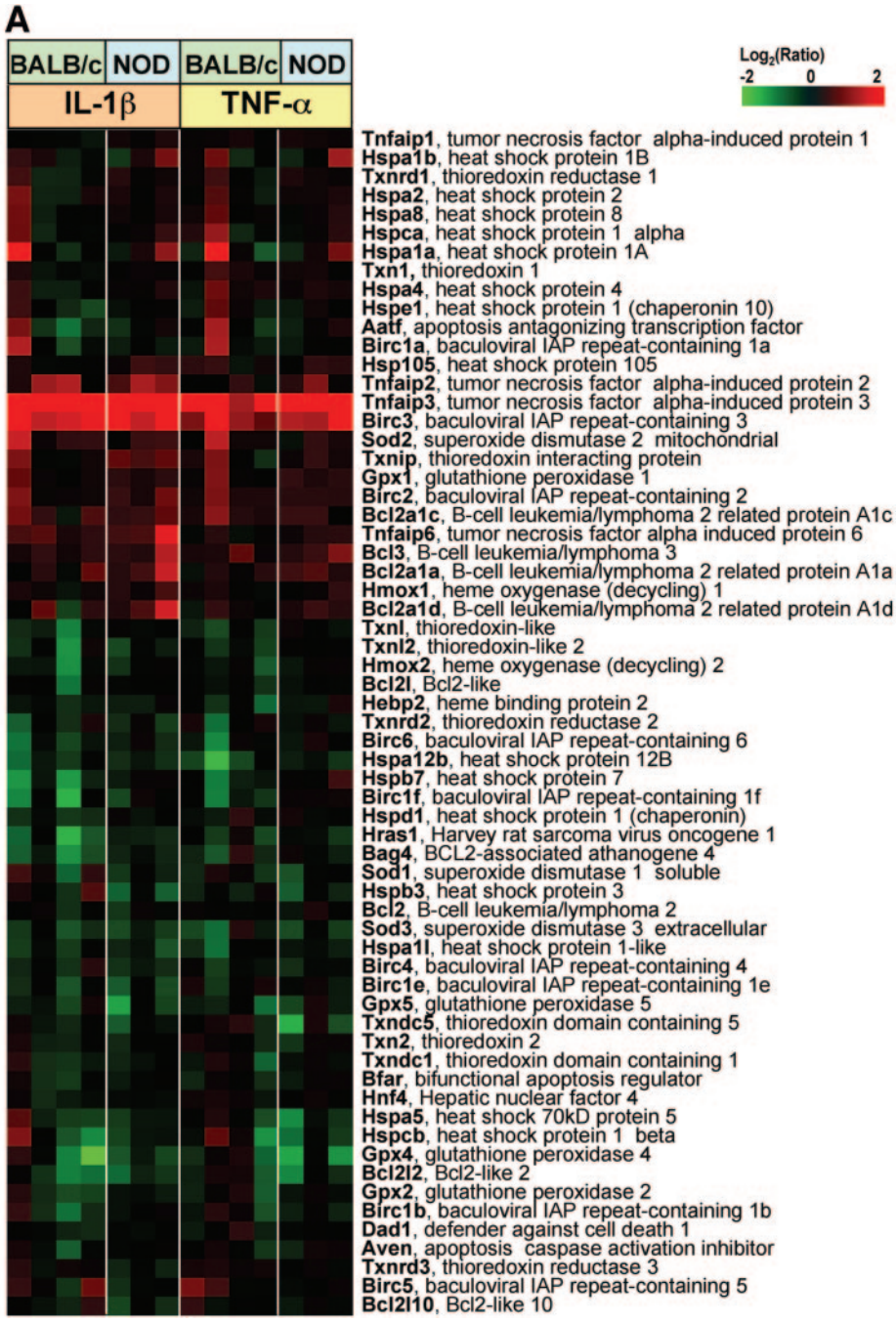

FIG. 2

data demonstrate β-cell–specific regulation of A20 expression within pancreatic islets.

Cytokine-dependent regulation of A20 requires de novo gene transcription. We next began to address the mechanism(s) by which A20 was regulated in islets. Primary rodent islets were pretreated with actinomycin D to block de novo gene transcription and then stimulated with IL-1β for 1 h. Analysis of A20 steady-state mRNA revealed that induction of A20 was completely abolished (>90%, $P \leq 0.001$) by actinomycin D treatment, indicating that in islets, cytokine-dependent induction of A20 is regulated at the level of de novo gene transcription (Fig. 5*A*). To confirm that A20 was regulated at the level of transcription, β-TC_3 cells were transfected with an A20 promoter sequence upstream of a luciferase reporter and stimulated with IL-1β or TNF-α. As shown in Fig. 5*C*, IL-1β and TNF-α induced approximately fourfold ($P \leq 0.001$) and approximately twofold ($P \leq 0.01$) increases in luciferase activity, respectively. Together these data demonstrate that A20 is regulated at the level of gene transcription in β-cells, and prevention of A20 expression by inhibition of gene transcription correlated with sensitization to TNF-α–induced apoptosis.

NF-κB is both necessary and sufficient to initiate transcriptional activation of the A20 promoter. A20 has been described previously to be an NF-κB target gene and contains two NF-κB binding sequences in its promoter region (42). To determine whether or not the induction of A20 transcription in β-cells is NF-κB dependent, we transfected β-TC_3 cells with an A20 promoter construct in

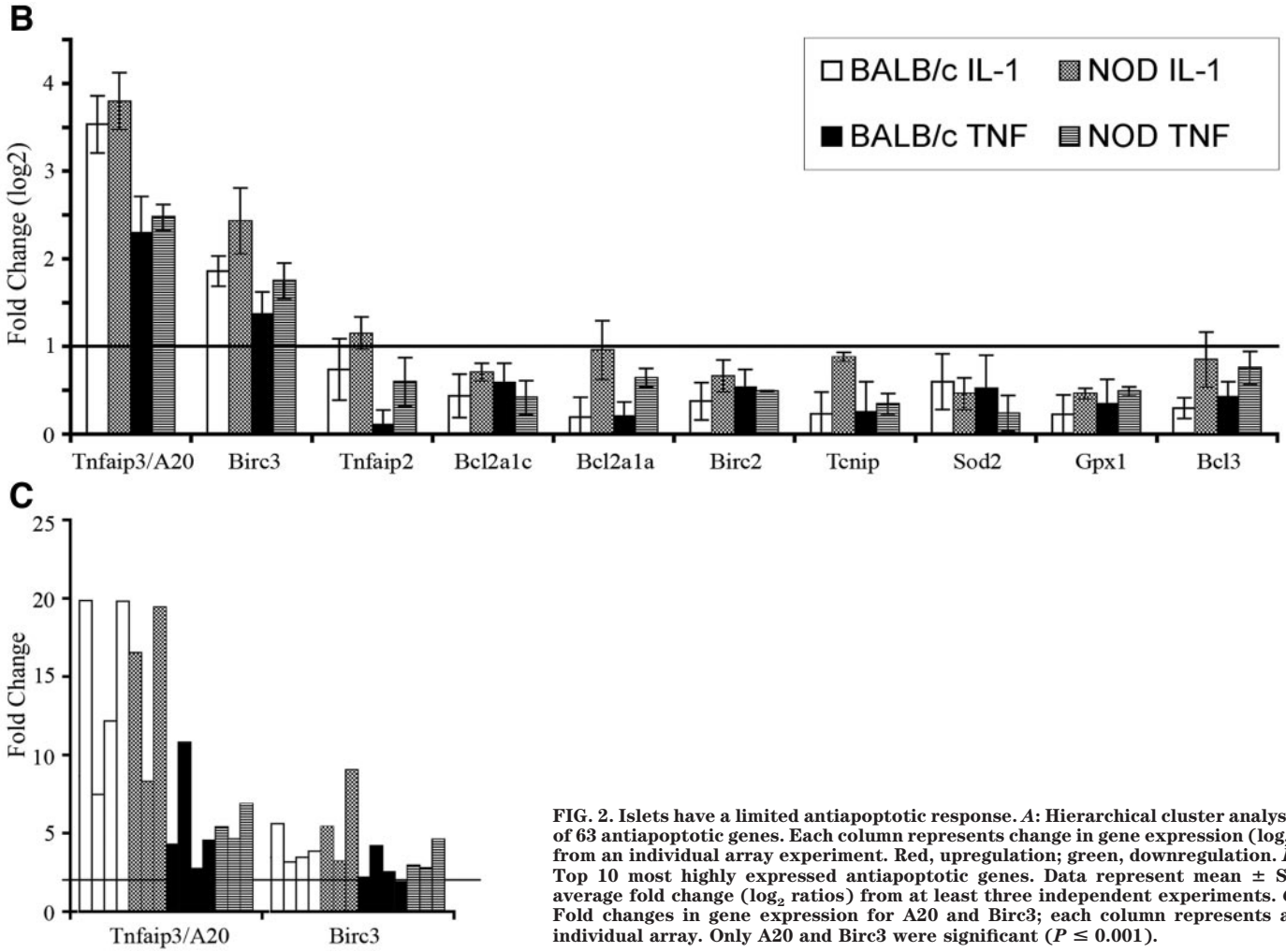

FIG. 2. Islets have a limited antiapoptotic response. *A*: Hierarchical cluster analysis of 63 antiapoptotic genes. Each column represents change in gene expression (log_2) from an individual array experiment. Red, upregulation; green, downregulation. *B*: Top 10 most highly expressed antiapoptotic genes. Data represent mean ± SD average fold change (log_2 ratios) from at least three independent experiments. *C*: Fold changes in gene expression for A20 and Birc3; each column represents an individual array. Only A20 and Birc3 were significant ($P \leq 0.001$).

which the two NF-κB binding sites were deleted (ΔNF-κB) (Fig. 5*B*) and examined its activation in response to either IL-1β or TNF-α. As shown in Fig. 5*C*, compared with the wild-type promoter, both the cytokine-dependent and constitutive A20 promoter activities were completely abrogated ($P \leq 0.001$) in the NF-κB–deleted reporter.

We next utilized an alternative approach to confirm the importance of NF-κB in the regulation of de novo transcription of the A20 promoter. We achieved this by overexpressing the p65/RelA subunit of NF-κB (39) in β-TC_3 cells and examined whether this change would result in induction of the A20 promoter. As shown in Fig. 6*B*, forced expression of p65/RelA resulted in a marked dose-dependent activation of the A20 promoter, showing an ~19-fold increase ($P \leq 0.001$) at the highest concentration tested. Importantly, forced expression of p65/RelA also resulted in a fivefold induction ($P \leq 0.005$) in endogenous A20 mRNA levels, indicating that NF-κB was sufficient to drive the endogenous A20 promoter (Fig. 6*A*). Finally, inhibition of NF-kB activity using the chemical inhibitor, pyrrolidine dithiocarbamate (28), abolished IL-1β–stimulated A20 mRNA (>90%, $P \leq 0.01$) expression in islets (Fig. 6*C*.) Together these data demonstrate that NF-κB is both necessary and sufficient to initiate transcriptional activation of the A20 promoter and drive expression of A20 mRNA. These data are consistent with our finding that A20 is regulated by the NF-κB–activating cytokines IL-1β and TNF-α, but not cytokines such as γ-IFN, IL-4, IL-13, and IL-15, which do not activate NF-κB.

A20 transcription is regulated by multiple NF-κB signaling pathways. NF-κB constitutes a family of transcription factors that together act as major integrators of the cellular inflammatory response (39,43). Activation of discrete NF-κB pathways through the Toll-like receptors (TLRs) or TNR receptors (TNF-Rs) or via free radicals results in the induction of a distinct sets of genes (39,41,44). To determine the important NF-κB pathways regulating A20 expression in β-cells, we utilized a transient transfection approach, cotransfecting β-TC_3 cells with the A20 reporter and specific kinases or adapter proteins, known to activate distinct NF-κB signaling pathways. TRAF6 is an adaptor protein required for NF-κB signaling through the TLR family, whereas TRAF2 mediates NF-κB signaling through the TNF-R family. As shown in Fig. 7*A* and *B*, forced expression of either TRAF6 or TRAF2 dose dependently activated the A20 promoter with ~9-fold ($P \leq 0.001$) and ~6.5-fold ($P \leq 0.001$) increases in reporter activity at the highest concentrations used, respectively. Thus, we demonstrate that A20 is a downstream target gene of the canonical NF-κB pathway, activated via ligation of TLR and TNF-R families. These data are consistent with our experiments demonstrating that increased A20 expression in IL-1β– (TLR-activating) and TNF-α– (TNF-R–activating) stimulated β-cells.

We next addressed whether A20 would be regulated in response to the noncanonical and free radical–dependent pathways. NIK is critical for mediating signaling through the noncanonical TNF-R pathway (39), whereas free rad-

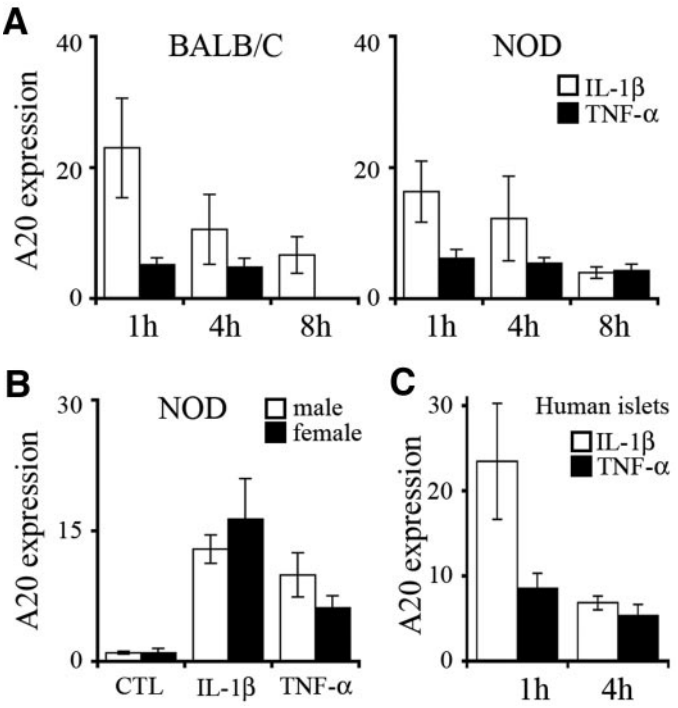

FIG. 3. A20 is an immediate early response gene in β-cells. *A*: A20 expression in BALB/c or NOD islets treated with IL-1β or TNF-α. Data represents means ± SD from three independent experiments. *B*: A20 expression in male or female NOD islets stimulated with IL-1β or TNF-α for 1 h. Data represents means ± SD from three independent experiments. *C*: A20 expression in primary human islets treated with IL-1β or TNF-α. Data represent means ± SD from four independent experiments. All differences are significant ($P \leq 0.05$).

icals activate NF-κB through a PKD-dependent signaling pathway (44). To determine whether A20 is a target gene of these pathways, we cotransfected β-TC_3 cells with the A20 promoter and either NIK or PKD (Fig. 7*C* and *D*). Forced expression of NIK resulted in an ~3.6-fold ($P \leq 0.005$) activation of the A20 promoter, whereas PKD induced a more modest ~2.6-fold ($P \leq 0.001$) induction of reporter activity. These data demonstrate that A20 transcription can be induced by TNF-Rs that activate the noncanonical pathway and by free radicals. Together our data identify A20 as a major NF-κB target gene regulated by diverse NF-κB signaling pathways.

Expression of A20 in stressed islets in vivo. Transplanted islets are exposed to a hyperglycemic environment, nonspecific inflammatory reactions (i.e., including cytokines and free radicals), and immune-mediated rejection as well as stresses relating to hypoxia and lack of adequate nutrients (10,11). Many of these factors are able to induce NF-κB. Having identified A20 as a major NF-κB–regulated gene in β-cells, we hypothesized that A20 expression should be heavily regulated in islets in vivo after exposure to inflammatory stress. To assess this directly, we examined A20 expression levels in islets after transplantation into either diabetic syngeneic or diabetic allogeneic hosts. As shown in Fig. 8, already at day 5 after transplantation, there were significant approximately threefold ($P \leq 0.05$) and approximately fourfold ($P \leq 0.05$) increases in A20 steady-state mRNA expression in islets transplanted into either syngeneic or allogeneic recipients, respectively. These data demonstrate that transplanted islets do have a regulated antiapoptotic stress response and that A20 is a critical component of this stress response in vivo.

A20 is sufficient to protect β-cells from TNF-α–induced cell death. We demonstrated that blocking de novo gene transcription sensitizes β-cells to apoptosis and prevents A20 upregulation, indicating that loss of A20 expression may be partly responsible for the sensitization to TNF-α–induced apoptosis. To determine the importance of A20 in regulating β-cell apoptosis, we next asked whether A20 expression was sufficient to protect β-cells from TNF-α–induced apoptosis.

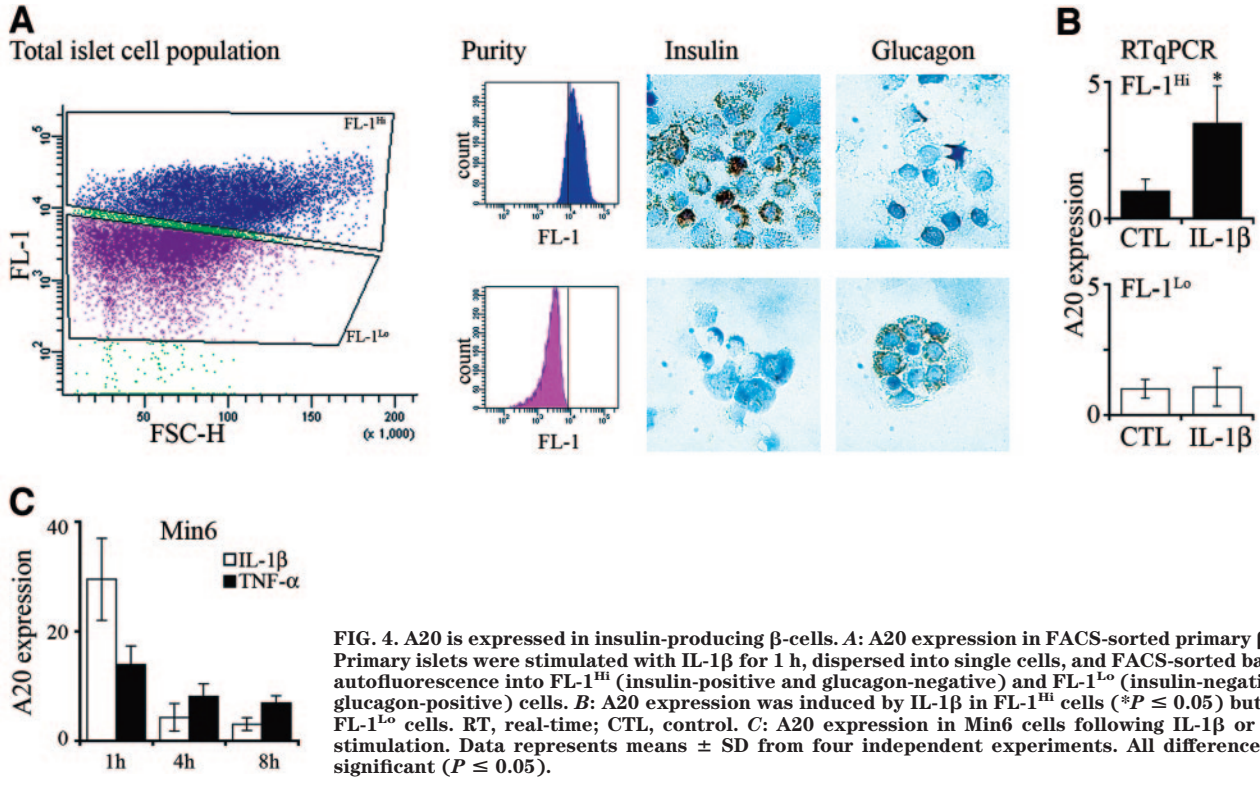

FIG. 4. A20 is expressed in insulin-producing β-cells. *A*: A20 expression in FACS-sorted primary β-cells. Primary islets were stimulated with IL-1β for 1 h, dispersed into single cells, and FACS-sorted based on autofluorescence into $FL\text{-}1^{Hi}$ (insulin-positive and glucagon-negative) and $FL\text{-}1^{Lo}$ (insulin-negative and glucagon-positive) cells. *B*: A20 expression was induced by IL-1β in $FL\text{-}1^{Hi}$ cells (*$P \leq 0.05$) but not in $FL\text{-}1^{Lo}$ cells. RT, real-time; CTL, control. *C*: A20 expression in Min6 cells following IL-1β or TNF-α stimulation. Data represents means ± SD from four independent experiments. All differences were significant ($P \leq 0.05$).

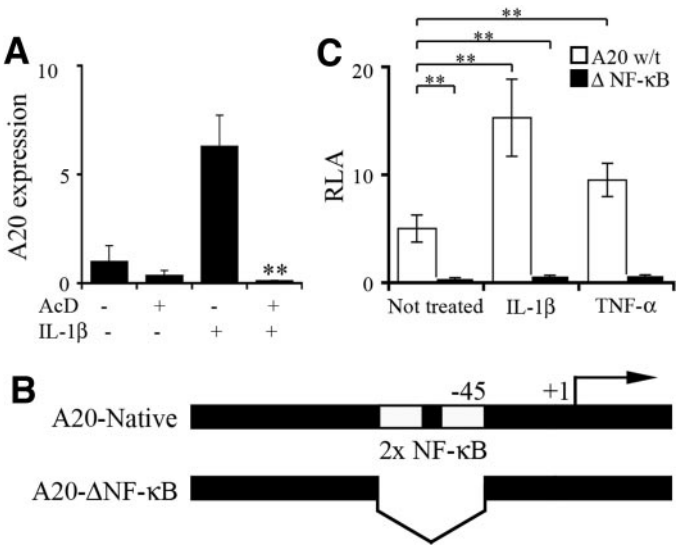

FIG. 5. A20 is regulated at the level of transcription by NF-κB. *A*: A20 expression in BALB/c islets treated with IL-1β for 1 h with or without actinomycin D (AcD). Data represent means ± SD from two independent experiments. *B*: A20 promoter sequence indicating deleted NF-κB binding sites used for reporter studies. *C*: Induction of the native or ΔNF-κB A20 reporter in β-TC_3 by IL-1β or TNF-α. Data are means ± SD from a representative experiment of three independent experiments. All differences are significant ($P \leq 0.001$).**

Activation of the TNF receptor by its cognate ligand results in the recruitment of FADD, which subsequently recruits and activates procaspase 8/FLICE, triggering apoptosis (1,39). For this experiment, β-TC_3 cells were transiently cotransfected with a FADD expression vector to induce apoptosis in the presence or absence of an A20 expression plasmid. Controls cells were transfected with a vector expressing a FADD dominant negative inhibitor (FADD-DN) or the empty vector $pcDNA_3$. All groups were

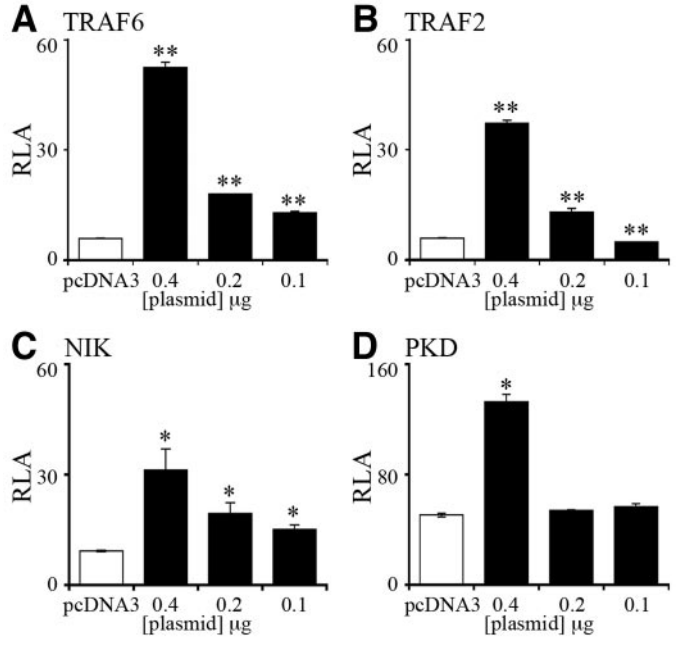

FIG. 7. A20 expression is NF-κB dependent in β-cells. Induction of the A20 reporter in β-TC_3 by (*A*) TRAF6, (*B*) TRAF2, (*C*) NIK, and (*D*) PKD. Data represent means ± SD from one representative experiment of four to six independent experiments conducted. All differences are significant (*$P \leq 0.05$; **$P \leq 0.001$). RLA, relative light activity.

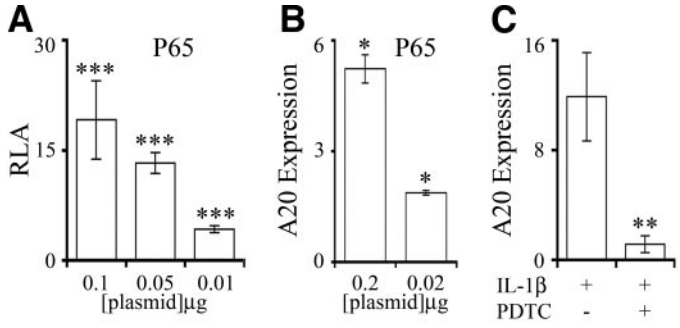

FIG. 6. NF-κB is necessary and sufficient to drive de novo A20 expression. *A*: Induction of the A20 reporter in β-TC_3 by p65/RelA. Data represent means ± SD from a representative experiment of three independent experiments. *B*: Induction of endogenous A20 mRNA in β-TC_3 by p65/RelA. Data represent means ± SD from a representative experiment of three independent experiments conducted. *C*: A20 expression in BALB/c islets treated with IL-1β for 1 h with or without pyrrolidine dithiocarbamate. Data represent means ± SD of three independent experiments. All differences are significant (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$). RLA, relative light activity; PDTC, pyrrolidine dithiocarbamate.

transiently cotransfected with a cytomegalovirus-driven β-gal reporter, which was used to determine the percentage of viable cells for each group (22). As shown in Fig. 9, forced expression of FADD resulted in the rapid destruction of β-TC_3 cells as evidenced by the decrease in β-gal reporter activity (~60%, $P \leq 0.005$) compared with baseline levels observed in control groups (e.g., $pcDNA_3$ or FADD-DN), whereas expression of FADD-DN had little effect upon cell viability ($P = 0.132$). In contrast, A20 expressing cells were completely protected from the proapoptotic effect of FADD ($P \leq 0.05$). These data demonstrate for the first time that A20 is sufficient to protect β-cells from TNF-α–induced apoptosis; thus, loss of A20 expression by transcriptional blockade sensitizes β-cells to apoptotic cell death.

DISCUSSION

Apoptotic β-cell death is an important pathological mechanism of β-cell loss in type 1 diabetes and islet graft rejection (5,6). Apoptosis is a highly regulated process, such that the decision to undergo apoptosis is dependent upon the balance between antiapoptotic and proapoptotic signals (19). In an inflammatory setting, cells are required to express a new or inducible set of antiapoptotic genes as a mechanism to counteract the physiological stresses that otherwise lead to cellular damage and apoptosis (19,39). Blockade of this regulated antiapoptotic response sensitizes many cell types to apoptotic death, underscoring the

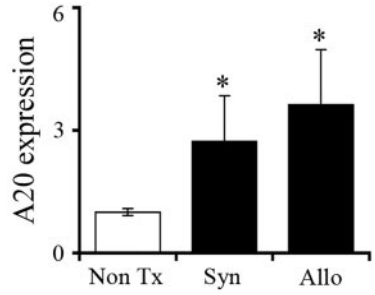

FIG. 8. A20 is upregulated in islets in vivo. Islet A20 expression at postoperative day 5. Data represent means ± SD from three independent experiments. All differences are significant (*$P \leq 0.05$). Non Tx, nontransplanted; Syn, syngeneic; Allo, allogeneic.

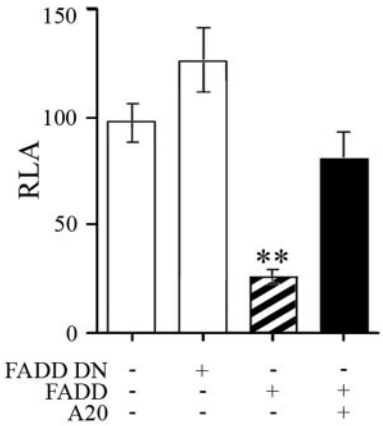

FIG. 9. A20 rescues β-cells from FADD-induced cell death. Cell survival in β-TC$_3$ cells expressing pcDNA3, FADD-DN, FADD, or FADD plus A20. Data represent mean ± SD percentage survival from four independent experiments. Differences between FADD and FADD plus A20 are significant ($P \leq 0.05$). RLA, relative light activity.**

physiological relevance of this signaling network in cell biology (16–18).

In this present study we demonstrate that this paradigm is true for β-cells, as β-cells were sensitized to TNF-α–induced death by inhibition of de novo gene transcription. Our microarray-based approach revealed that TNFAIP3/A20 is one potential candidate gene providing a molecular basis for this protective response. A20 is a zinc finger–containing, immediate early-response gene with a potent antiapoptotic and anti-inflammatory function (20,40,45). This essential antiapoptotic and anti-inflammatory function of A20 is preserved in islets (13,21). Once expressed, A20 binds to and targets TNF receptor interacting protein for proteosomal degradation, thereby preventing TNF-α–induced NF-κB activation (46). The antiapoptotic mechanism of A20 is less well understood but may also involve inhibition of key proximal signaling events, as A20 inhibits activation of initiator caspases after death receptor ligation (22), consistent with our data demonstrating that A20 protects β-cells from FADD-induced apoptosis.

The transcription factor NF-κB regulates multiple proinflammatory genes that can contribute to islet destruction. NF-κB can promote T-cell–mediated killing and the generation of β-cell toxins through the induction of molecules such as Fas (47,48), inducible nitric oxide synthase (48,49) and cyclooxygenase-2 (50), respectively. In addition, the promoters of other proinflammatory genes induced in β-cells, including chemokines (i.e., monocyte chemoattractant protein-1) and adhesion molecules (i.e., intercellular adhesion molecule-1), also possess binding elements for NF-κB (43). The importance of NF-κB in β-cell inflammatory responses is underscored by the fact that blockade of NF-κB in in vitro models, by means of an inhibitor of NFκB (IκBα) super-repressor or via A20 overexpression, prevents IL-1β– and γ-IFN–induced β-cell dysfunction and death (21,48,49,51). Collectively these data implicate NF-κB as a major player in cytokine-dependent β-cell dysfunction and indicate that targeting NF-κB may have therapeutic utility as a means to improve islet function in the face of inflammatory insults.

However, aside from its important proinflammatory role, NF-κB is a major regulator of cellular apoptosis through its ability to control the expression of multiple antiapoptotic genes including the c-IAPs, caspase-8-c-FLIP, Bfl-1/A1 (39), and A20 (20,42). In cells that lack the NF-κB family member p65/RelA, exposure to TNF-α will induce apoptosis (16). Furthermore, blockade of NF-κB activation, by nonspecifically inhibiting transcription with actinomycin D (52) or specifically via the use of an IκBα super-repressor (17,18), also sensitizes cells to TNF-α–mediated apoptosis. Importantly blockade of NF-κB in β-cells by means of an IκBα super-repressor (53) or via actinomycin D as we demonstrate here, also sensitizes cells to TNF-α–dependent apoptotic death. We propose that this sensitization was due to blockade of NF-κB activation and, at least in part, the subsequent loss of A20 expression. Consistent with this, prevention of A20 induction in vitro sensitizes endothelial cells to TNF-dependent apoptosis (20). Thus in β-cells, as has been demonstrated for other cell types, NF-κB activation governs both proinflammatory responses and protection from apoptosis (39). Therefore, targeting NF-κB activation in an in vivo setting in which multiple factors are at play may sensitize β-cells to apoptosis (53). In light of this, we propose that more sophisticated approaches than mere blanket blockade of NF-κB are warranted if one aims to prevent the unwanted proinflammatory effects of NF-κB activation without incurring the associated risk of sensitizing β-cells to apoptotic death. Alternative strategies might include expressing genes such as A20 (13,20) or targeting specific upstream regulators of NF-κB activation (54).

In conclusion, we demonstrate that primary islets upregulated a relatively small set of antiapoptotic genes in response to inflammatory stress, of which TNFAIP3/A20 was the most highly regulated. Here we demonstrate that A20 is regulated at the level of gene transcription in pancreatic β-cells, under the control of the transcription factor NF-κB; thus, tightly linking islet proinflammatory gene responses with protection from apoptosis. Together with our previous studies demonstrating an anti-inflammatory and antiapoptotic function for A20 in islets (13,21), these present data indicate that A20 is a critical component of the islet-regulated response to inflammatory stress and injury. Thus, pathologic loss of A20 expression may render β-cells susceptible to apoptotic death; conversely, enhancing A20 expression in β-cells may improve their survival in the face of inflammatory and autoimmune insults. Importantly, our data indicate that blockade of NF-κB, as a means to prevent islet inflammatory responses in vivo, may have the unwanted side effect of sensitizing β-cells to apoptosis by preventing the upregulation of antiapoptotic genes such as A20 (16,17,20).

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