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*J Immunol* published online 5 June 2013
http://www.jimmunol.org/content/early/2013/06/05/jimmunol.1300344

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Synergistic Expression of the CXCL10 Gene in Response to IL-1β and IFN-γ Involves NF-κB, Phosphorylation of STAT1 at Tyr<sup>701</sup>, and Acetylation of Histones H3 and H4

Susan J. Burke,* Matthew R. Goff,* Danhong Lu, † David Proud, ‡ Michael D. Karlstad,*§ and J. Jason Collier*§

The CXCL10 gene encodes a peptide that chemoattracts a variety of leukocytes associated with type 1 and type 2 diabetes. The present study was undertaken to determine the molecular mechanisms required for expression of the CXCL10 gene in response to IL-1β and IFN-γ using rat islets and β cell lines. IL-1β induced the expression of the CXCL10 gene and promoter activity, whereas the combination of IL-1β plus IFN-γ was synergistic. Small interfering RNA-mediated suppression of NF-κB p65 markedly inhibited the ability of cytokines to induce the expression of the CXCL10 gene, whereas targeting STAT1 only diminished the synergy provided by IFN-γ. Furthermore, we found that a JAK1 inhibitor dose dependently reduced IFN-γ–controlled CXCL10 gene expression and promoter activity, concomitant with a decrease in STAT1 phosphorylation at Tyr<sup>701</sup>. We further discovered that, although the Tyr<sup>701</sup> phosphorylation site is inducible (within 15 min of IFN-γ exposure), the Ser<sup>727</sup> site within STAT1 is constitutively phosphorylated. Thus, we generated single-mutant STAT1 Y701F and double-mutant STAT1 Y701F/S727A adenoviruses. Using these recombinant adenoviruses, we determined that overexpression of either the single- or double-mutant STAT1 decreased the IFN-γ–induced expression of CXCL10 plus IFN-γ plus IFN-β expressed by rat islets and 293T cells. Furthermore, we found that a JAK1 inhibitor dose dependently reduced IFN-γ–mediated CXCL10 gene expression and promoter activity, whereas the combination of IL-1β plus IFN-γ was synergistic. Small interfering RNA-mediated suppression of NF-κB p65 markedly inhibited the ability of cytokines to induce the expression of the CXCL10 gene, whereas targeting STAT1 only diminished the synergy provided by IFN-γ. Furthermore, we found that a JAK1 inhibitor dose dependently reduced IFN-γ–controlled CXCL10 gene expression and promoter activity, concomitant with a decrease in STAT1 phosphorylation at Tyr<sup>701</sup>. We further discovered that, although the Tyr<sup>701</sup> phosphorylation site is inducible (within 15 min of IFN-γ exposure), the Ser<sup>727</sup> site within STAT1 is constitutively phosphorylated. Thus, we generated single-mutant STAT1 Y701F and double-mutant STAT1 Y701F/S727A adenoviruses. Using these recombinant adenoviruses, we determined that overexpression of either the single- or double-mutant STAT1 decreased the IFN-γ–mediated potentiation of CXCL10 gene expression, promoter activity, and secretion of protein. Moreover, the Ser<sup>727</sup> phosphorylation was neither contingent on a functional Tyr<sup>701</sup> site in β cells nor was it required for cytokine-mediated expression of the CXCL10 gene. We conclude that the synergism of IL-1β and IFN-γ to induce expression of the CXCL10 gene requires NF-κB, STAT1 phosphorylated at Tyr<sup>701</sup>, recruitment of coactivators, and acetylation of histones H3 and H4.

The Journal of Immunology, 2013, 191: 000–000.

Diabetes mellitus occurs when a reduction in functional pancreatic β cell mass is severe enough to produce hyperglycemia. This decrease in islet β cell function and mass occurs through organ-specific autoimmunity in type 1 diabetes (1, 2) and is strongly correlated with obesity-associated tissue dysfunction in type 2 diabetes (3, 4). A commonality between both of these endocrine diseases is the increase in pancreatic β cell production of soluble factors that, once secreted, operate as chemotactic cytokines (hereafter referred to as chemokines). Chemokines are secreted peptides that recruit immune cells to the site of inflammation (5, 6). The chemokines are grouped according to placement of an N-terminal cysteine residue within the peptide, leading to assignment to the C, C-C, C-X-C, or C-X3-C families. Approximately 50 discrete chemokines have been identified, corresponding to ~20 distinct chemokine receptors (reviewed in Refs. 6, 7). The expression of specific chemokine genes in the pancreatic β cell is increased in both rodents and humans prior to and during diabetes (8–11); therefore, it is possible that islet β cells are the very source of the soluble factors that initiate an influx of immune cells that contribute to their demise. The C-C and C-X-C chemokines are the largest families and contain some of the most well-studied chemoattractive peptides, including CCL2 and CXCL10. CXCL10 expression is elevated in pancreatic islets isolated from individuals with type 1 or type 2 diabetes (12, 13). Additionally, CXCL10 circulates at higher levels in diabetic patients than in nondiseased individuals (8, 14, 15). Transgenic expression of CXCL10 driven by pancreatic β cell–specific promoters revealed islets enriched with specific leukocytes (10, 16). An increase in the macrophage, T lymphocyte, and other immune cell populations results in an insulitis (islet inflammation) that leads to the release of proinflammatory cytokines, such as IL-1β and IFN-γ. Pancreatic β cell exposure to IL-1β and IFN-γ leads to accumulation of intracellular mediators of inflammation that suppress insulin secretion (17, 18), increased expression of genes that contribute to the inflammatory response (19–22), and, eventually, a decrease in functional β cell mass (23–25). Although IL-1β and IFN-γ clearly activate signaling pathways culminating in pancreatic β cell death and dysfunction, little is known about the mechanisms and associated protein factors that control gene expression of secreted proteins that potentially create a feed-forward amplification of this vicious inflammatory cycle.

IL-1β signals through the NF-κB pathway to regulate the expression of a variety of genes involved in inflammation. NF-κB is a dimer of proteins that includes RelA/p65, RELB, c-Rel, p50, and p52. p65 is held in the cytoplasm by a group of regulatory proteins, referred to as IκBs. Phosphorylation-induced degradation of IκBα by IκB kinases unmasks a nuclear-localization signal within p65, allowing it to be translocated into the nucleus (26). Nuclear p65 acts as either a homodimer or heterodimer with p50 to activate gene transcription. In addition, stimuli that activate NF-κB, such as IL-1β, are often potentiated by concurrent activation of the IFN-γ–signaling pathway (22, 27). However, mechanisms re-

*Department of Nutrition, University of Tennessee, Knoxville, TN 37996; †Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham, NC 27704; ‡Department of Physiology and Pharmacology, University of Calgary, Calgary, Alberta T2N 4Z6, Canada; and §Department of Surgery, Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN 37920

Received for publication February 4, 2013. Accepted for publication April 25, 2013.

Address correspondence and reprint requests to Dr. J. Jason Collier, Department of Nutrition, University of Tennessee, Knoxville, 1215 W. Cumberland Avenue - 229 JHB, Knoxville, TN 37996-1920. E-mail address: Jason.collier@utk.edu

Abbreviations used in this article: ChIP, chromatin immunoprecipitation; CTD, carboxyl-terminal domain; DM, double mutant; IκBα, IκB-α-galactosidase; IκBαΔN, IκBα superrepressor; ISE, IFN-stimulated response element; siRNA, small interfering RNA; 3xGAS, three copies of the consensus γ-activated sequence.

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sensible for the IFN-γ-mediated potentiation of NF-κB–driven gene transcription in pancreatic β cells are not well understood. IFN-γ signals through a cell surface receptor to activate STAT proteins to control transcriptional outputs (28). STAT1 is phosphorylated at Tyr701 and Ser727, and these sites are often responsible for maximal transcriptional activity in response to a specific stimulus. However, the requirement for STAT1 and associated phosphoacceptor sites in the cytokine-mediated regulation of the CXCL10 gene in pancreatic β cells has not been established. Therefore, we undertook the current study to investigate the signals, genomic response elements, and primary DNA-binding factors responsible for controlling expression of the CXCL10 gene by IL-1β and IFN-γ in insulin-producing cells. Several key discoveries were made: the CXCL10 gene is activated by IL-1β, a response that is synergistic with concomitant activation of the IFN-γ–signaling pathway; the proximal and distal IFN-stimulated response elements (ISRE) within the CXCL10 promoter were required for the activation by both IL-1β and IFN-γ; the proximal and distal IFN-stimulated response elements (ISRE) within the CXCL10 promoter were required for signal integration by both IL-1β and IFN-γ; the proximal ISRE is activated by IL-1β, and primary DNA-binding factors responsible for controlling expression of the CXCL10 gene by IL-1β were required for the activation by both IL-1β and IFN-γ; the proximal and distal ISRE: (F) 5′-GAGAGTTGGTAGG-TGC-3′ and (R) 5′-GTCAACCTCTTGGCAGGCTAT-3′. All mutations were generated using the QuikChange Site-Directed Mutagenesis kit, according to the manufacturer’s recommended protocol, and verified by dideoxy sequencing prior to construction of recombinant adenoviral vectors. Recombinant adenoviruses were generated by cotransfection of the pAC.CMV STAT1 shuttle vectors and pm17 viral genome into HEK293 cells, as previously documented (38). All adenoviruses were confirmed to be insert positive and E1A negative, prior to recommendations (39).

### Materials and Methods

**Cell culture, islet isolation, and reagents**

The 832/13 and INS-1E cell lines are derived from the parental INS-1 cell line (29). Culture and passage of the INS-1–derived 832/13 and INS-1E cell lines were described (30, 31). Islets were isolated from Wistar rats per a previous report (19). IL-1β was purchased from Thermo Fisher Scientific, IFN-γ was from Shenandoah Biotechnology, and JAK inhibitor was from EMD Millipore. The three copies of the consensus γ-activated sequence (3xGAS)–luciferase reporter plasmids were a gift from Dr. Thomas Spencer, Washington State University, Pullman, WA (32). Recombinant adenoviruses expressing β-galactosidase (β-GAL) (33), wild-type STAT1 and Y701F STAT1 (20), IκBα superrepressor (34), and p65 wild-type (35) have been described. Silencer Select small interfering RNA (siRNA) oligonucleotides were purchased from Life Technologies.

### Plasmid construction and site-directed mutagenesis

pGL3-luciferase vectors containing approximately −1 kb and −400 bp of the human CXCL10 promoter, in addition to NF-κB (distal and proximal) and proximal ISRE mutations in the −1 kb promoter, were described previously (36). Luciferase reporter vectors containing five copies of the distal κB element or five copies of the proximal κB element from the CXCL10 gene promoter were also described (37). The aforementioned CXCL10 −1 kb construct was used to generate mutations in the distal ISRE, whereas the CXCL10 −400 bp construct was used to construct a proximal ISRE mutation by site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis kit, according to the manufacturer’s instructions (Agilent Technologies). The following primer pairs were used to incorporate these mutations: distal ISRE: (F) 5′-CATCGTTGAATCACTCCTTGTTCGGCTCA-GTCTACTTTC-3′ and (R) 5′-GAGAGTTGGTAGGACGAC-ACAAGGATGGATCTGACGATG-3′; proximal ISRE: (F) 5′-CAGTGGACTTGGACTTTTGGAATC-3′ and (R) 5′-GTGATTAGTGTTCTCAGTCATGGGAAA-3′.

### Transfection and luciferase assay

Transient transfections of luciferase reporter constructs into 832/13 cells were achieved using TransIT-LT1 transfection reagent (Mirus Bio). siRNA oligonucleotides (siRNA ID for p65: s159517; siRNA ID for STAT1: s129044; negative control siRNA: catalog no. M4611) were transfected using DharmFECT Transfection Reagent 1 (Thermo Scientific). Concomitant transfection of plasmid and siRNA was performed using Dharm aFECT Duo (Thermo Scientific). Whole-cell lysates were prepared, and luciferase activity was measured, as previously described (19).

### Recombinant adenoviruses

pAC.CMV STAT1 (a gift from Dr. Guoxun Chen, University of Tennessee, Knoxville) was used as a template to generate the S727A and S727T mutations in STAT1. Recombinant adenovirus expressing STAT1 Y701F mutant (20) was described previously, and the pAC.CMV shuttle vector containing the Y701F mutation was used as a template to generate the Y701F/S727A double mutation. Primer pairs used to incorporate mutations in STAT1 were as follows: S727A: (F) 5′-GAAACCTGTTCATGGTCTCAG-3′ and (R) 5′-GTCAACCTCTTGGCAGGCTAT-3′; S727T: (F) 5′-GAAACCTGTTCATGGTCTCAG-3′ and (R) 5′-GTCAACCTCTTGGCAGGCTAT-3′. All mutations were generated using the QuikChange Site-Directed Mutagenesis kit, according to the manufacturer’s recommended protocol, and verified by dideoxy sequencing prior to construction of recombinant adenoviral vectors. Recombinant adenoviruses were generated by cotransfection of the pAC.CMV STAT1 shuttle vectors and pm17 viral genome into HEK293 cells, as previously documented (38). All adenoviruses were confirmed to be insert positive and E1A negative, prior to recommendations (39).

### RNA isolation, synthesis of cDNA, and mRNA expression analysis

Total RNA isolation, cDNA synthesis, and gene-expression analysis via real-time RT-PCR were performed according to previous protocols (19). Primer sequences for RSP9, p65, E1A, STAT1, and CXCL10 were generated using Primer3Plus software and are available upon request.

### Protein isolation and immunoblotting

Whole-cell lysates were prepared using M-PER lysis solution supplemented with a protease/phosphatase inhibitor mixture (both from Thermo Fisher Scientific). Proteins were quantified by bicinchoninic acid assay (Thermo Fisher Scientific), separated on a 4–12% SDS-PAGE gel (Invitrogen), and transferred to polyvinylidene difluoride membranes. Membranes were blocked with Membrane Blocking Solution (Invitrogen) for 45 min prior to primary Ab incubation in 1% polyvinylpyrrolidone (24, 40). Membranes were stripped and reprobed with each Ab in the order shown in the respective figures (top to bottom). Abs used were from the following sources: tubulin, POA-Y701 STAT1, POA-S727 STAT1, and total STAT1 (Cell Signaling) and β-actin (Sigma-Aldrich).

### Chromatin immunoprecipitation

832/13 cells were cultured to confluence in 10-cm dishes, using one dish/treatment condition. After treatment of cells, media were aspirated, and formaldehyde was added at a final concentration of 1% (diluted in PBS) and incubated at room temperature for 10 min. Glycine was added to a final concentration of 125 mM for 5 min at room temperature. Cells were washed once with cold PBS and scraped into 1 ml prechilled PBS with 1× protease inhibitors (Halt Protease Inhibitor Cocktail; Thermo Scientific). Cell pellets were collected by centrifugation at 2000 rpm for 2 min at 4°C. Supernatants were discarded, and pellets were resuspended in 0.5 ml Sds Lysis Buffer containing 1× protease inhibitors (1% SDS, 10 mM EDTA, 50 mM Tris-HCl [pH 8]). Lysates were incubated on ice for 15 min, followed by DNA sonication at 4°C with a Misonix Sonicator S-4000 (Thermo Scientific). The following sonication conditions generated fragments of DNA between 100 and 500 bp in length: Amplitude: 4; Process Time: 10 min, Pulse on: 10 s, Pulse off time: 20 s. Sonicated fragments were centrifuged at 12,000 × g for 10 min at 4°C, and supernatants were transferred to a clean, low-rotation microcentrifuge tube. Preclearing was performed for 1 h at 4°C with rotation using 5 μl magnetic Protein G beads (Thermo Scientific). Once beads were removed, 100 μl sheared cross-linked DNA was diluted 10-fold in dilution buffer containing 1× protease inhibitors (0.01% SDS, 1% Triton X-100, 1.2 mM EDTA, 167 mM NaCl, 17 mM Tris [pH 8]). Two micromotors of immunoprecipitating Abs were added and incubated overnight at 4°C with rotation. Immunoprecipitation of DNA–protein–Ab complexes was performed by incubation with 10 μl Protein A (for rabbit polyclonal Abs) or Protein G (for mouse monoclonal antisera) Dynabeads (Invitrogen) for 1 h at 4°C. The magnetic beads binding to the Ab–Ag–chromatin complexes were washed sequentially with 0.5 ml the following buffers: 1× Low-Salt Buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris [pH 8]), 1× High-Salt Buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl, 20 mM Tris [pH 8]), 1× LiCl Buffer (0.25 M LiCl, 1% Nonidet P-40, 1 mM EDTA, 10 mM Tris [pH 8]), and 2× Tris-EDTA. Once the last wash buffer (Tris-EDTA) was completely removed, 100 μl 10% Chelex 100 resin in PBS was added, vortexed for 10 s, and heated to 100°C for 10 min. Following centrifugation at 17,000 × g for 1 min at 4°C, the supernatants were transferred to new tubes. A total of 120 μl 1× phosphate-free water was added, vortexed, and centrifuged. Supernatants were pooled. Inputs were processed as followed: 20 μl sonicated, precleared DNA was incubated overnight at 65°C with NaCl to a final concentration of 200 mM. Input DNA was then treated with RNase A for...
30 min at 37°C and proteinase K for 1 h at 45°C, followed by purification using a QIAGEN Cleanup Kit (QIAGEN). A total of 2.5 μg DNA was used as a template for downstream SYBR Green–based PCR reactions. Fold enrichment of promoter fragments was calculated using the ΔΔΔCT method: 2(CΔΔC control immunoprecipitation − CΔΔC experimental immunoprecipitation). All data are shown as the fold chromatin immunoprecipitation (ChIP) signal over the IgG background signal. Negative control regions corresponding to cAMP response element–binding regions of the NRA2 gene were used to confirm transcription factor binding specificity. Abs were from the following sources: normal rabbit and mouse IgG were from Sigma Aldrich; p65, p50, CBP, p300, and STAT1 were from Santa Cruz Biotechnology; histone acetyl H3 K9, PO4-Ser2 RNA polymerase II, and PO4-Ser5 RNA polymerase II were from Abcam; and histone acetyl H4 K5, 8, 12, 16 and total RNA polymerase II were from Millipore. Primer sequences for amplifying the CXCL10 proximal and distal NF-κB and ISRE sites were generated using Primer3Plus software and are available upon request.

ELISA

Secretion of CXCL10 into the media was detected using the Rat IP-10 (CXCL10) Mini ELISA Development Kit (PeproTech), according to the suggested protocol. CXCL10 release into the media was normalized to total protein to account for any potential differences in cell number.

Statistical analysis

One-way ANOVA, followed by the Tukey post hoc correction, or Student t test was used to identify statistical significances at the confidence levels of 90, 95, and 99%, as reported within the figure legends.

Results

Expression of the CXCL10 gene is increased by IL-1β and IFN-γ in rat islets and β cell lines

CXCL10 levels are elevated in individuals with diabetes mellitus (41, 42), and expression of CXCL10 has been colocalized to the β cells of the pancreatic islets (13). Thus, we investigated the signals that increase the expression of the CXCL10 gene using rat islets and β cell lines. We found that 1 ng/ml IL-1β induced the CXCL10 gene by ~47-fold in 832/13 rat insulinoma cells. After setting this 47-fold IL-1β response at 100%, we discovered that 100 U/ml IFN-γ potentiated the IL-1β response by an additional 8-fold (Fig. 1A). A similar response was obtained in the INS-1E cell line (Fig. 1B). IL-1β and IFN-γ individually enhanced steady-state CXCL10 mRNA levels in isolated rat islets, and the combination of these cytokines synergistically augmented expression of the CXCL10 gene (Fig. 1C). To further examine the transcriptional responses, we next transfected 832/13 cells with a plasmid containing ~1 kb of the CXCL10 proximal gene promoter (upstream of the transcriptional start site), which includes known κB and ISRE sequences, driving a luciferase reporter gene. We found that IL-1β increased promoter activity by 9-fold (set at 100% in Fig. 1D), whereas IFN-γ alone did not augment transcription over baseline. However, the combination of IL-1β plus IFN-γ induced a synergistic 27-fold cumulative response (shown as 3-fold potentiation of the IL-1β response; Fig. 1D). We further note that CXCL10 protein was secreted from 832/13 β cells in response to IL-1β and that this secretory response was further amplified by the addition of IFN-γ + IL-1β (Fig. 1E). Thus, the ability of IL-1β and IFN-γ to induce synthesis and secretion of CXCL10 is due, at least in part, to increased transcription of the CXCL10 gene by these proinflammatory cytokines.

Deletion and mutational analysis of the CXCL10 gene promoter reveals the importance of κB and ISRE sequences controlling gene transcription in response to cytokines

We next examined the transcriptional response to IL-1β and IFN-γ, alone and in combination, using either ~1 kb or ~400 bp constructs of the CXCL10 proximal gene promoter. The ~1 kb genomic sequence containing two NF-κB response elements was 8-fold responsive to 1 ng/ml IL-1β (IL-1β response set at 100%; Fig. 2A). IFN-γ alone did not activate the promoter (Fig. 1D), but it did potentiate the response to IL-1β by an additional 3.3-fold (Fig. 2A). Mutating either the distal or proximal κB element led to an 89.4 and 67.8% decrease, respectively, in the ability of IL-1β to induce transcription of the promoter luciferase reporter gene. Additionally, the ability of IFN-γ to potentiate the response to IL-1β was also diminished by ~50% (1.5- and 1.6-fold potentiation for distal and proximal mutations, respectively).

Because of the differential magnitude of transcriptional effects observed after mutating the distal versus proximal κB response element on the cytokine response, we used multimerized response elements to individually examine transcriptional activities in both the basal (unstimulated) and inducible states. We discovered that the distal κB element drove almost a 2-fold greater transcriptional response in the basal state compared with the proximal κB element (Fig. 2B). In contrast, when each individual κB construct was exposed to a concentration range of IL-1β, the proximal κB element was revealed to have the most robust response to IL-1β (56.6-fold induction of the proximal κB element versus 26.2-fold induction of the distal κB element in response to 0.1 ng/ml IL-1β; Fig. 2C). This observation remained consistent, regardless of IL-1β concentration.

We next examined the cytokine responsiveness of a CXCL10 promoter–luciferase deletion construct, which contains 400 nucleotides upstream of the transcriptional start site but does not include the distal ISRE element. In these experiments, we found that the proximal ISRE site was largely responsible for the IFN-γ–mediated potentiation of the IL-1β response, because deletion of the 600 nucleotides upstream (containing the distal ISRE site) had minimal effects on the IL-1β responsiveness or the ability of IFN-γ to potentiate the IL-1β response (Fig. 2D).

To further investigate the observations made using the ~400 kb promoter, we mutated the distal and proximal ISRE sites individually and in combination within the ~1 kb gene promoter construct. Using these promoter constructs, we found that mutations in the proximal ISRE element eliminated both IL-1β– and IFN-γ–mediated gene activation (Fig. 2E). Moreover, although the distal site mutant also markedly impaired IL-1β activation (94% decrease relative to wild-type), the potentiation by IFN-γ was maintained (2.8-fold in wild-type versus 3.1-fold in distal ISRE mutant; Fig. 2E), albeit with a diminution in the magnitude of the response. Mutations in both the distal and proximal ISRE elements completely abolished transcription of the ~1 kb CXCL10 gene promoter in response to IL-1β and IFN-γ (Fig. 2E). Therefore, despite both κB sites being intact, the ISRE elements contain nucleotide information required to confer overall signal integration at the CXCL10 gene promoter in response to IL-1β. Furthermore, although both ISRE sites clearly participate in regulation of the IL-1β response, the proximal site most likely is the key regulator of the IFN-γ–mediated potentiation.

p65/RelA and STAT1 are required for activation of the CXCL10 gene by cytokines

After determining the genomic response elements required for IL-1β– and IFN-γ–mediated activation of the CXCL10 gene (Fig. 2), we next examined which primary DNA-binding factors were necessary for gene activation. Because p65 and STAT1 were the two most likely candidates to activate κB and ISRE elements, respectively, we used two strategies for this approach: overexpression of the IκBα superrepressor (IκBαSR) and sRNA-mediated targeting of either the p65/RelA subunit of NF-κB or STAT1. The IκBαSR protein contains S32A/S36A mutations that prevent phosphorylation-induced degradation and, thus, promote cytoplasmic retention of p65 (34). Overexpression of the IκBαSR in 832/13 cells completely blocked the cytokine-mediated induction of the CXCL10 gene (Fig. 3A), which indicates involvement of NF-κB proteins and
is consistent with the requirement of κB sites to confer transcriptional responsiveness by cytokines (Fig. 2A). In addition, IκBαSR diminished the cytokine-mediated secretion of CXCL10 (49% decrease relative to βGAL; Fig. 3B), confirming that NF-κB-mediated increases in transcription also augment protein production and release. To directly examine p65 subunit involvement, we next used siRNA-directed suppression and found that expression of the p65 transcript was decreased by 75.4% by sip65, whereas the STAT1 transcript was diminished by 91.3% with siSTAT1 duplex transfection (Fig. 3C). The reduction in mRNA corresponding to the p65 and STAT1 genes produced a decrease in total p65 and STAT1 protein (Fig. 3D). Using these specific siRNA approaches, we discovered that the IL-1β–stimulated expression of the CXCL10 gene was impaired by 74% (Fig. 3E, white bars); in contrast, the potentiation of the IL-1β response by IFN-γ was unaffected (5.7-fold versus 5.6-fold; Fig. 3F, black bars). Alternatively, depletion of STAT1 by siRNA duplex transfection decreased the IFN-γ–mediated potentiation by 28.7% (12.2-fold versus 8.7-fold potentiation, Fig. 3F, black bars), but it did not impair the CXCL10 response to IL-1β (Fig. 3F, white bars).

p65, STAT1, and RNA polymerase II are recruited to the CXCL10 gene promoter in a signal-specific manner

Using ChIP assays, we examined the occupancy of the key regulatory factors p65 and STAT1 at the CXCL10 gene promoter in
response to IL-1β or IFN-γ. We detected p65 occupancy at the CXCL10 κB sites in the basal state (Fig. 4A, white bar), with a 130% increase in response to IL-1β (Fig. 4A, black bars).

An increase in promoter-bound p50, the typical heterodimer partner for p65, was also detected at κB sites, and IL-1β stimulation produced a 109% increase in ChIP signal (Fig. 4B). Consistent with IFN-γ potentiating the effects of IL-1β, we detected a 6.86-fold increase in STAT1 ChIP signal in response to this cytokine within 15 min, which diminished to 2.49- and 1.94-fold at 30 and 60 min, respectively (Fig. 4C).

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We next examined whether p300 or CBP, known coactivators of p65 (43, 44), were recruited to the CXCL10 gene promoter in response to IL-1β. There was a 181% increase in p300 at the κB sites within 15 min of IL-1β stimulation (Fig. 4D), consistent with the kinetics of p65 occupancy. CBP was present at high levels in the basal state, which matches the CXCL10 gene having a relatively high baseline expression level. In addition, IL-1β promoted an increase in CBP ChIP signal from 6.04-fold to 14.72-fold.

**FIGURE 3.** p65/RelA and STAT1 are required for activation of the CXCL10 gene by cytokines. (A) 832/13 cells were transduced with recombinant adenoviruses expressing either βGAL or IκBα S32A/S36A (IκBαSR). At 24 h posttransduction, cells were stimulated for 6 h with either 1 ng/ml IL-1β or IL-1β plus 100 U/ml IFN-γ. *p < 0.05 versus βGAL with IL-1β, **p < 0.05 versus βGAL with IL-1β + IFN-γ. (B) 832/13 cells were transduced with the indicated adenoviruses. At 24 h posttransduction, cells were left untreated or were stimulated for 12 h with 1 ng/ml IL-1β plus 100 U/ml IFN-γ. CXCL10 release into the media was quantified by ELISA. **p < 0.01. (C and D) 832/13 cells were transfected with siRNA duplexes against either p65 or STAT1 with a negative control siScramble. (C) Expression of p65 and STAT1 was quantified by RT-PCR. (D) STAT1 and p65 abundance was measured via immunoblot with β-actin as a loading control. 832/13 cells were transfected with siRNA duplexes targeting either p65 (E) or STAT1 (F), with a negative scrambled sequence as a control for specificity (siScramble). At 48 h posttransfection, cells were stimulated with either 1 ng/ml IL-1β or IL-1β plus 100 U/ml IFN-γ for 6 h. In (E): *p < 0.05 versus siScramble with IL-1β. (F): *p < 0.05 versus siScramble with IL-1β + IFN-γ. In (A), (E), and (F), total RNA was isolated, and the mRNA level of CXCL10 was normalized to that of RS9. 832/13 cells were transfected concomitantly with CXCL10 2 kb and siRNA duplexes targeting either p65 (G) or STAT1 (H). At 24 h posttransfection, cells were stimulated with IL-1β alone or in combination with IFN-γ for 4 h prior to measurement of luciferase activity. Values are mean ± SEM from three or four individual experiments. **p < 0.01 versus siScramble with IL-1β, **p < 0.01 versus siScramble with IL-1β + IFN-γ.
(144% increase; Fig. 4D). This accumulation of regulatory proteins at the CXCL10 gene promoter correlated with an approximate doubling in the acetylation of both histones H3 and H4 in response to IL-1β (Fig. 4E). Moreover, there was a 59.24-fold increase in the recruitment of total RNA polymerase II to the gene promoter (Fig. 4F). We also found a 3.1-fold increase in Ser\(^\tau\) phosphorylation of the carboxyl-terminal domain (CTD) of RNA polymerase II at the core promoter at 15 min, with further increases at 60 min (Fig. 4G) and a 3.71-, 4.96-, and 6-fold enhancement of Ser\(^\tau\) phosphorylation of the RNA polymerase II CTD on the coding region after 15, 30, and 60 min of IL-1β exposure, respectively (Fig. 4H). These changes are consistent with a gene undergoing transcriptional activation (45) and, to our knowledge, represent the first report of such alterations for the CXCL10 gene in response to proinflammatory cytokines in β cells.

Thus, we conclude that the synergistic increase in the expression of the CXCL10 gene by IL-1β and IFN-γ is through the docking of the key regulatory proteins NF-κB and STAT1 at the genomic response elements in the proximal promoter region, associated recruitment of the coactivators p300 and CBP, and alterations in the local chromatin environment (i.e., increases in acetylation of histones H3 and H4). These changes at the CXCL10 gene promoter likely assist in recruitment of RNA polymerase II and promote signal-induced modifications to the

**FIGURE 4.** p65, STAT1, and RNA polymerase II are recruited to the CXCL10 gene promoter in a signal-specific manner. (A–C) 832/13 cells were stimulated with either 1 ng/ml IL-1β or 100 U/ml IFN-γ for 0, 15, 30, and 60 min. ChIP assays were performed using Abs against p65 (A), p50 (B), or STAT1 (C) and normalized to assays performed using control IgG Ab. The recovered CXCL10 NF-κB and ISRE genomic regions were amplified by SYBR Green–based RT-PCR. (D) 832/13 cells were incubated with 1 ng/ml IL-1β for 15 min. ChIP assays were conducted using Abs against p65, p300, and CBP. (E–H) 832/13 cells were stimulated with 1 ng/ml IL-1β for 15, 30, or 60 min. ChIP assays were performed using Abs against acetylated histone H3 and H4 (E), total RNA polymerase II (F), polymerase II CTD-PO\(_{\tau}\)-Ser5 (G), or polymerase II CTD-PO\(_{\tau}\)-Ser2 (H). In (A–F), the recovered CXCL10 genomic regions targeted for amplification via real-time RT-PCR are shown above each individual graph. Data are mean ± SEM from three independent experiments. \( * p < 0.05 \) versus corresponding NT groups (A–D, F); \( * p < 0.1 \) versus the other corresponding NT groups, \( * p < 0.05 \) versus the intragroup comparison (G). \( ** p < 0.01 \) versus corresponding NT groups. n.s., Not significant versus NT.
enzyme that allows transcriptional activation of the CXCL10 gene.

Pharmacological inhibition of JAK1 blocks the IFN-γ-mediated potentiation of IL-1β–stimulated CXCL10 gene expression

Because the maximal expression of the CXCL10 gene requires both IL-1β and IFN-γ, with IFN-γ augmenting the IL-1β response, we investigated whether phosphorylation of STAT1 was required for this process. First, we observed that IFN-γ induced an early (within 15 min) phosphorylation of STAT1 at Tyr701 (Fig. 5A). Interestingly, Ser727 was constitutively phosphorylated in the pancreatic β cell (Fig. 5A), with no attendant increase in response to IFN-γ. Similar results were obtained using INS-1E cells (data not shown) and in isolated rat islets (Fig. 5B). These results are in contrast to other tissues in which Ser727 is an inducible site and is dependent on prior phosphorylation of Tyr701 (46). Next, we found that titrating in increasing amounts of a JAK1 inhibitor led to impairments in phosphorylation of Tyr701 by IFN-γ (Fig. 5C). At the highest dose used (100 nM), we also noticed a decrease in Ser727 phospo-signal (Fig. 5C). The decrease in phospho-STAT1 at Tyr701 correlated with a concentration-dependent reduction in transcriptional activation of a synthetic luciferase reporter gene driven by 3xGAS (Fig. 5D). Furthermore, IFN-γ–mediated potentiation of the CXCL10 gene in the presence of the JAK1 inhibitor was also diminished by 59% at the highest concentration (Fig. 5E). We also observed similar results using the −1 kb proximal CXCL10 gene promoter, with 100 nM JAK1 inhibitor decreasing the IFN-γ potentiation by 42% (Fig. 5F). Thus, the ability of IFN-γ to augment IL-1β–mediated transcription requires JAK1 activity, which most likely signals through the inducible Tyr701 phosphorylation of STAT1.

FIGURE 5. Pharmacological inhibition of JAK1 blocks the IFN-γ–mediated potentiation of IL-1β–stimulated CXCL10 gene expression. 832/13 cells (A) and rat islets (B) were stimulated with 100 U/ml IFN-γ for the indicated times. Whole-cell lysates were extracted and blotted for phospho-STAT1 (Y701 and S727) and total STAT1 abundance. The abundance of tubulin (A) and β-actin (B) serves as loading control. (C) 832/13 cells were pretreated with increasing concentrations (0.1, 1, 10, or 100 nM) of a JAK inhibitor (JAKi) for 1 h, followed by 15 min of stimulation with 100 U/ml IFN-γ. Whole-cell lysates were extracted and analyzed for phospho-STAT1 (Y701 and S727) and total STAT1 abundance. The abundance of tubulin (A) and β-actin (B) serves as loading control. (D) 832/13 cells were transfected with a 3xGAS-luciferase construct; at 24 h posttransfection, cells were pretreated for 1 h with increasing concentrations of JAKi (1, 10, or 100 nM), followed by a 4-h stimulation with 100 U/ml IFN-γ. *p < 0.05 versus IFN-γ with DMSO. (E) 832/13 cells were transfected with CXCL10 −1 kb promoter-luciferase construct. At 24 h posttransfection, the cells were treated with 1, 10, or 100 nM JAKi for 1 h, followed by a 4-h stimulation with 1 ng/ml IL-1β or IL-1β plus 100 U/ml IFN-γ. In (D) and (F), promoter luciferase activity was measured. Data are mean ± SE from three independent experiments. *p < 0.05 versus DMSO with IL-1β + IFN-γ.
Inhibition of signaling through JAK1 blocked the ability of IFN-γ to potentiate the IL-1β–stimulated expression of the CXCL10 gene (Fig. 5). Because Tyr701 is a major phospho-acceptor site activated in response to IFN-γ (Fig. 5A) (47), we generated a recombinant adenovirus that encodes STAT1 with a Y701F mutation. As expected, the mutant protein is not phosphorylated upon cellular exposure to IFN-γ (Fig. 6A). However, the constitutive phosphorylation of STAT1 at Ser727 in the pancreatic β cell was still present in the Y701F mutant STAT1 protein (Fig. 6A), indicating the phospho-acceptor sites are not dependent on each other for their individual activation. We note that the STAT1 Y701F has much less activity than does wild-type STAT1 on a synthetic 3xGAS-luciferase reporter gene, despite retaining constitutive Ser727 phosphorylation (Fig. 6B). Additionally, the IFN-γ–mediated potentiation of the CXCL10 gene was reduced by 39 and 51%, in the presence of increasing concentrations of STAT1 Y701F protein (Fig. 6C). Moreover, we discovered that, although wild-type STAT1 is capable of enhancing the IFN-γ–stimulated increase in promoter activity using the −1 kb CXCL10 luciferase reporter, STAT1 Y701F did not retain this ability (Fig. 6D). Further, cytokine-dependent secretion of CXCL10 protein was diminished by 43% in the presence of overexpressed STAT1 Y701F (Fig. 6E). Thus, phosphorylation of STAT1 at Tyr701 is required for maximal activation of the CXCL10 gene and release of protein in response to IL-1β + IFN-γ.

Conservative or loss-of-function mutations at the Ser727 phospho-acceptor site do not block IFN-γ–mediated increases in expression of the CXCL10 gene

The serine phosphorylation of STAT1 is required for the expression of many genes in a variety of tissues (48). Because the Ser727 site in STAT1 is constitutively phosphorylated in the pancreatic β cell and does not require prior phosphorylation at Tyr701, we generated recombinant adenoviruses that express either STAT1 with S727A (loss-of-function) or S727T (conservative) mutations. In Fig. 7A, it is clear that phosphorylation of STAT1 at Ser727 was diminished, albeit with different magnitudes, in these mutant proteins. The removal of the phospho-acceptor site (S727A) reduced the ability of STAT1 to support transcription from a 3xGAS-luciferase reporter gene (Fig. 7B). In contrast, the conservative change of serine to threonine, which retains the hydroxyl group that serves as a phospho-acceptor site, displayed activity similar to the wild-type STAT1 on a 3xGAS reporter gene (Fig. 7C). Despite the STAT1 S727A mutant having decreased activity on a synthetic STAT1-regulated reporter gene, this mutant STAT1 did not display any loss in activity when we examined cytokine-mediated CXCL10 gene expression (Fig. 7D). Moreover, the S727T mutant was also perfectly capable of supporting a response to the CXCL10 gene in response to IL-1β and IFN-γ (Fig. 7E). Thus, despite phosphorylation of Ser727 being required for expression of cytokine-responsive genes in other tissues (49, 50) and for activation of a consensus multimerized γ-activated sequence element in the pancreatic β cell (Fig. 7B), the CXCL10 gene does not require phosphorylation at Ser727 within STAT1 to support its expression.

To test the hypothesis that Ser727 synergizes with Tyr701 upon the latter’s inducible phosphorylation to activate transcription, we next examined how a double-mutant (DM) Y701F/S727A mutant impacted expression of the CXCL10 gene. The abundance of wild-type and DM STAT1 are shown in Fig. 8A, with the clear inability to augment phosphorylation at the mutated sites in response to cytokine stimulus. The decrease in phosphorylation at both of the two key regulatory sites in STAT1 resulted in impairment in the ability to activate the 3xGAS-luciferase reporter gene (Fig. 8B). Moreover, expression of the CXCL10 gene was dose dependently diminished by 53.6, 64.6, and 74.7% with increasing concentrations of STAT1 DM protein (Fig. 8C). In the isolated rat islet, the STAT1 DM displayed comparable activity, with a 54% decrease in expression of the CXCL10 gene in response to IL-1β + IFN-γ (Fig. 8D). In addition, promoter activity was reduced by 60.8% and secretion of CXCL10 protein was reduced by 30.1% with overexpression of the DM STAT1 protein (Fig. 8E, 8F). These data are incredibly similar to those seen with the single Y701F mutation and, therefore, support the lack of necessity for Ser727 phosphorylation to drive expression of the CXCL10 gene in pancreatic β cells. Thus, we conclude that maximal induction of the CXCL10 gene by IL-1β and IFN-γ in pancreatic β cells requires a functional phospho-acceptor site at Tyr701 within the STAT1 protein to communicate signals induced by IFN-γ.

Discussion

CXCL10 peptide levels are elevated in the blood of individuals with type 1 diabetes mellitus (8, 11, 12, 14) and are associated with rejection of transplanted islets (51, 52). Synthesis and secretion of CXCL10 from the pancreatic β cell occur in both type 1 and type 2 diabetes mellitus (13), and forced expression from pancreatic β cells of transgenic mice accelerates immune-mediated diabetes (10). Thus, a thorough understanding of the signals and factors required to enhance expression of the CXCL10 gene could be beneficial for developing strategies to diminish recruitment of proinflammatory stimuli–induced leukocyte populations. In the current study, we demonstrated a strong synergy between IL-1β and IFN-γ to induce the expression of the CXCL10 gene. To our knowledge, these data are the first to show the molecular regulation of the CXCL10 gene in response to proinflammatory cytokines in pancreatic β cells.

We specifically note the involvement of what appears to be a cooperative set of κB and ISREs. Normally, κB sites respond via NF-κB–activating stimuli (e.g., IL-1β, TNF-α), whereas γ-activated sequence/ISRE elements respond to type I and/or type II IFNs. In the case of IL-1β and IFN-γ signaling to the CXCL10 gene in pancreatic β cells, the κB and ISRE sites appear to be operating concurrently to fine tune the response of each signal in a cooperative manner (Figs. 1, 2). This type of signal integration at the genomic level offers the potential to alter the expression of the CXCL10 gene in response to proinflammatory cytokines in pancreatic β cells.
current study provides such information regarding the CXCL10 gene. Additional examples of genes other than chemokines whose expression is also strongly controlled by proinflammatory cytokines in pancreatic β cells include those encoding inducible NO synthase (22, 56) and PG-endoperoxide synthase 2 (aka cyclooxygenase-2) (20, 57). Delineating precise complexes required for transcription of genes controlling inflammatory processes, such as those that encode chemokines and other proteins involved in inflammatory responses, will be required to understand how inflammation is managed at the molecular level.

Along these lines, we demonstrated previously that the CCL2 gene is highly responsive to IL-1β but is not potentiated by IFN-γ in pancreatic β cells (19), despite IFN-γ responsiveness in other tissues (58). The distinction in signal integration at given gene promoters, such as seen with CCL2 versus CXCL10, may be one way to help orchestrate a cautious recruitment of quantities and types of immune cells by carefully titrating amounts of chemotactic proteins made and secreted. The synergistic nature of IFN-γ amplifying the response to IL-1β to modulate CXCL10, but not CCL2, expression fits with this interpretation. In addition, TNF-α and IFN-γ synergize to induce the expression of CXCL10 gene in human airway smooth muscle cells (59), indicating a global importance for understanding the regulation of this gene and its role in a broad spectrum of inflammatory processes. However, no increase in acetylation of his-
tone H3 was observed in airway smooth muscle cells in response to TNF-\(\alpha\) or IFN-\(\gamma\), indicating tissue-specific and/or signal-specific regulatory strategies to control gene transcription.

As part of our molecular analysis, we discovered a tissue-specific phosphorylation pattern of STAT1 in the \(\beta\) cell that is distinct from other cell types. The pancreatic \(\beta\) cell constitutively phosphorylates STAT1 at Ser\(^{727}\), regardless of whether the Tyr\(^{701}\) site is intact. This is in contrast to other tissues, which were shown to require Tyr\(^{701}\) phosphorylation as a prerequisite for Ser\(^{727}\) phosphorylation (46, 49). Thus, the phosphorylation patterns of STAT1 could partially help to explain the potentiation of the IL-1\(\beta\) response by IFN-\(\gamma\), with activation of the inducible Tyr\(^{701}\) site assisting in promotion of a multiregulatory complex that enhances the transactivation capacity of primary DNA-binding factors and/or recruitment of RNA polymerase II. Interestingly, in the context of the CXCL10 gene promoter, whether Ser\(^{727}\) is phosphorylated or not does not appear to impact transcription of this gene in pancreatic \(\beta\) cells (Fig. 7D).

FIGURE 7. Amino acid substitutions at the Ser\(^{727}\) phospho-acceptor site do not block IFN-\(\gamma\)-mediated increases in expression of the CXCL10 gene. (A) 832/13 cells were transduced with adenoviruses encoding \(\beta\)GAL, STAT1, or two increasing concentrations of STAT1 with either S727A or S727T mutations. Whole-cell lysates were immunoblotted for phospho-STAT1 (Y701 and S727) and total STAT1 abundance, with \(\beta\)-actin serving as a loading control (left panel). Densitometric analysis of the immunoblot displays the relative ratio of phosphorylated STAT1 (S727) to total STAT1 (right panel). Note the decrease in phospho-STAT1 in the mutants. 832/13 cells were transfected with a 3xGAS-luciferase construct; at 4 h posttransfection, cells were transduced with adenoviruses expressing \(\beta\)GAL, STAT1, or two increasing concentrations of either STAT1 S727A (B) or STAT1 S727T (C). Following overnight culture with the indicated adenoviruses, the cells were incubated for 4 h with 100 U/ml IFN-\(\gamma\). Promoter activity was quantified and normalized to protein content via BCA assay. (D and E) 832/13 cells were transduced with the indicated recombinant adenoviruses; at 24 h posttransduction, cells were treated for 6 h with 1 ng/ml IL-1\(\beta\) alone or IL-1\(\beta\) plus 100 U/ml IFN-\(\gamma\). Total RNA was extracted, and mRNA abundance of CXCL10 was quantified via real-time RT-PCR. The concentrations of adenoviruses used in (B)–(E) are identical to those shown in (A). Data shown are mean \(\pm\) SEM from three independent experiments. **\(p\) < 0.01 versus STAT1.
**FIGURE 8.** A DM STAT1 at Y701F/S727A reveals that the Tyr701 site predominantly controls the response to IFN-γ. (A) 832/13 cells were exposed for 24 h to adenoviruses expressing βGAL, STAT1, or STAT1 DMs (mutations Y701F and S727A are both present). Following 15 min of stimulation with 100 U/ml IFN-γ, whole-cell lysates were extracted and blotted for phospho-STAT1 (Y701 and S727) and total STAT1 abundance, with β-actin as a loading control. (B) 832/13 cells were transfected with a 3xGAS-luciferase construct; at 4 h posttransfection, cells were exposed to adenoviruses expressing βGAL, STAT1, or STAT1 Y701F/S727A. After overnight transduction with the indicated adenoviruses, cells were stimulated for 4 h with 100 U/ml IFN-γ. *p < 0.05 versus STAT1. 832/13 cells (C) and rat islets (D) were treated with the indicated adenoviruses; at 24 h posttransduction, cells were stimulated for 6 h with either 1 ng/ml IL-1β alone or IL-1β plus 100 U/ml IFN-γ (C) or 10 ng/ml IL-1β plus 100 U/ml IFN-γ (D). Total RNA was isolated and CXCL10 transcript levels were analyzed. **p < 0.01 versus βGAL with IL-1β + IFN-γ. (E) 832/13 cells were transfected with CXCL10 − 1 kb plasmid, followed by an 18-h exposure to the indicated adenoviruses and a subsequent 4-h incubation with 1 ng/ml IL-1β alone or IL-1β plus 100 U/ml IFN-γ. CXCL10 release into the media was quantified via ELISA and normalized to total cellular protein content. Data are mean ± SEM from three or four independent experiments. *p < 0.05 versus βGAL with IL-1β + IFN-γ. (G) IL-1β + IFN-γ synergistically increase transcription of the CXCL10 gene, thereby augmenting release of CXCL10 protein. A major part of this response includes constitutive phosphorylation of STAT1 at Ser727 and inducible phosphorylation (by IFN-γ) at Tyr701. Secreted CXCL10 promotes chemotaxis of immune cells via activation of the CXCR3 receptor and/or activates β cell TLR4 receptors in an autocrine/paracrine fashion. Dashed arrow indicates putative pathway activation.
Moreover, the S727T STAT1 also displayed no loss in function at either a 3xGAS reporter gene (Fig. 7C) or in the context of the CXCL10 gene promoter (Fig. 7E), which could indicate that the kinase responsible for constitutive activation of the Ser727 site within STAT1 is a Ser/Thr kinase. We further note that we could not detect a direct association of p65 with STAT1 via coimmunoprecipitation (S.J. Burke, M.R. Goff, & J.J. Collier, unpublished observations) as an explanation for synergy between IL-1β and IFN-γ, even though both proteins are clearly required for maximal expression of the CXCL10 gene (Figs. 1–3), and each individual protein is detected at the relevant cognate DNA binding sites using ChIP assays (Fig. 4). Taken together, the binding data and differential STAT1 phosphorylation patterns may offer insights into the control of the CXCL10 gene by inflammatory signals. Phosphorylation at Ser727 within STAT1 is dispensable for full expression of the CXCL10 gene; however, potentiation of the IL-1β response by IFN-γ is contingent on both JAK1 activity (Fig. 5) and tyrosine phosphorylation at the 701 position in STAT1 (Fig. 6).

Soluble secreted CXCL10 is a strong chemoattractant for T lymphocytes (60, 61); autoimmune destruction of islet β cells leading to type 1 diabetes is due, in large part, to a massive infiltration of T lymphocytes into the pancreatic islets (62, 63). Thus, enhanced secretion of CXCL10 directly from β cells exposed to proinflammatory signals, such as IL-1β and IFN-γ, may be a key contributing factor to diabetes development (64). Moreover, the increase in secreted CXCL10 protein driven by the combination of IL-1β and IFN-γ may also directly participate in β cell death and dysfunction by signaling through TLR4 on the β cell surface (Fig. 8F) (13). This fits with our own results that β cells do not express appreciable amounts of the CXCR3 protein (data not shown), which normally recognizes CXCL10 and other chemokines (e.g., CXCL9 and CXCL11) as ligands (65).

It is plausible that resident macrophages, which provide enough IL-1β to alter gene-expression patterns in the pancreatic β cells (22), in concert with infiltrating T cells combine to target insulin-positive cells for autoimmune-mediated destruction. This is fitting with extant studies demonstrating that T cell clones isolated from NOD mice immunized with insulin B-chain peptide fragments accelerated diabetes in neonatal mice, whereas the GAD65 (another autoantigen) T cell clone did not display this phenotype (66). A key distinction between these two T cell populations is that the clone immunized against the insulin B-chain expressed high levels of CXCL10 (66), whereas the other clones tested did not. Thus, synthesis and secretion of CXCL10 from specific T cell populations, in addition to that secreted from pancreatic β cells, would keep infiltrating T cells from leaving the islets and likely exacerbate accumulation of these and other immune cells. Moreover, CCL2 release from pancreatic β cells in response to IL-1β would promote recruitment of additional monocytes and macrophages into the islets (9, 19), in addition to contributing to T lymphocyte movement toward islet β cells (67). Taken together, these observations may help to explain the transition from peri-insulitis to invasive insulitis observed in NOD mice (68).

Therefore, attempts to design novel therapeutics for treatment of diabetes mellitus will benefit from a thorough understanding of how the β cell integrates signals, such as those elicited by cell surface receptors recognizing cytokine and chemokine ligands, to coordinate control transcriptional pathways regulating key soluble secreted factors linked to immune cell infiltration. Our present study has provided new knowledge regarding the phosphorylation state of STAT1 within pancreatic β cells, in addition to providing critical insights into the regulation of the CXCL10 gene by NF-κB and STAT1 in response to IL-1β and IFN-γ. It is possible that if a small molecule were designed to be able to selectively reduce CXCL10 signaling, this strategy would likely dampen the ability of the β cell to participate in its own demise through chemotactic and immune cell–modulatory activities. Interference with CXCL10 synthesis, secretion, or receptor interaction may also effectively reduce signaling through innate immune receptors, such as TLR4 (13). This approach may also be more selective than glucocorticoid-based therapies, which broadly target inflammation and have a host of undesirable side effects. A further understanding of proinflammatory cytokine–mediated control of chemokine gene networks will be required to facilitate improvement of therapeutic approaches to treat diseases associated with pathological inflammation.

Acknowledgments

We thank Drs. GuoXun Chen, Naima Moustaid-Moussa, Christopher Newgard, Thomas Spencer, and Jay Whelan for reagents. We also thank Emily Lazek for technical assistance.

Disclosures

The authors have no financial conflicts of interest.

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