Interleukin-1β activates a short STAT-3 isoform in clonal insulin-secreting cells

Nicholas M. Morton\textsuperscript{a}, Rolf P. de Groot\textsuperscript{b}, Michael A. Cawthorne\textsuperscript{a}, Valur Emilsson\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}Clore Laboratory, The University of Buckingham, Hunter Street, Buckingham MK18 1EG, UK
\textsuperscript{b}Department of Pulmonary Diseases, University Hospital Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

Revised 13 November 1998

Abstract Interleukin-1β (IL-1β) is a potent inflammatory cytokine involved in type 1 diabetes and acts through defined IL-1β signaling pathways. In the present work we describe induction of DNA binding activity to signal transducer and activator of transcription (STAT) in response to IL-1β in clonal insulin-secreting cells. Moreover, IL-1β activates a short isoform of STAT-3 that potently stimulates transcription. Immunoprecipitation studies reveal an interaction between the activated STAT-3 and the IL-1 receptor accessory protein indicating an association between the two signaling pathways. This leads to abrogation of glycolytic flux \cite{11}, mitochondrial and inflammatory mediators due to elevated expression of damage occurs after increased production of nitric oxide \cite{12,13,14}.

Key words: Interleukin 1 receptor accessory protein; Signal transducer and activator of transcription 3; Clonal β-cell; Interleukin 1 receptor accessory protein

1. Introduction

Interleukin-1β (IL-1β) is a potent pro-inflammatory cytokine that signals through the type I interleukin 1 receptor (IL-1RI) \cite{1,2}. IL-1RI and its essential co-receptor accessory protein (IL-1RACP) \cite{3} belong to the immunoglobulin superfamily of receptors and are distinct from other cytokine receptors \cite{2}. IL-1β is recognised as one of the instrumental factors in pancreatic islet β-cell destruction during autoimmune pancreatitis and type 1 diabetes \cite{4}. IL-1β mediates the intracellular activation through the IL-1R1-associated kinases, death domain proteins, the spondylosynovium/ceramide cascade, the nuclear factor NF-xB, Jun N-terminal kinase and mitogen-activated protein kinase signaling pathways \cite{1,5,8}. Beta-cell damage occurs after increased production of nitric oxide and inflammatory mediators due to elevated expression of inducible nitric oxide synthase \cite{9} and cyclooxygenase 2 \cite{10}. This leads to abrogation of glycolytic flux \cite{11}, mitochondrial respiratory processes \cite{12} and apoptotic cell death \cite{13,14}.

The Janus kinase (JAK) and signal transducer and activators of transcription (STAT) signaling pathway is associated with cytokines and growth factors that signal through the type I cytokine receptor superfAMILY \cite{15-17}. To date, there is little evidence suggesting cross talk between the IL-1β and JAK/STAT signaling pathways. There are no obvious JAK or STAT binding motifs within the IL-1RI or the IL-1RACP, and this could indicate that adapter proteins are necessary to couple these two pathways. In the present study we provide evidence for the activation of a short isoform of STAT-3 in response to IL-1β in the pancreatic β-cell model RINm5F. Furthermore, we find an interaction of the IL-1RACP with STAT-3 that supports a rapid recruitment and activation of the JAK/STAT pathway after binding of IL-1β to the IL-1RI/IL-1RACP complex. Since the susceptibility of the pancreatic β-cell to IL-1β-mediated damage is influenced by its metabolic status \cite{18,19}, we also determined the effect of mimicking increased metabolic activity on this signaling process. We propose that this novel point of cross talk between two distinct cytokine signaling pathways may be an additional mechanism used by IL-1β during the process of pancreatic β-cell destruction in type 1 diabetes.

2. Materials and methods

2.1. Cell culture and transient transfection

RINm5F cells were routinely cultured as described previously \cite{20}. Cells were exposed to recombinant human IL-1β (Calbiochem, Nottingham, UK), glucagon-like peptide-1 (GLP-1) (Bachem, Saffron Walden, UK), or forskolin and isobutyl methyl xanthine (IBMX) (Alexis Corp., Nottingham, UK) for the indicated times and concentrations in serum-free RPMI 1640 medium. For transfection experiments, interleukin-1γ-activated sequence (GAS)-chloramphenicol acetyltransferase (CAT) containing four copies of the human FeRl GAS (5’-AGC TTA GGA TGT ATT TTC CAG AAA GAG-3’), interleukin-1α-activated sequence (IRF-1)-CAT containing four copies of the human interleukin-1α response element 1 (ICAM-1)-CAT (5’-AGC TTA GGT TCC GGG AAA CCA G-3’), and β-casein-CAT containing four copies of the β-casein STAT binding site (5’-AGC TTA GTT TCC GGA TAC AAA TCA-3’) were used. STAT binding sites were cloned into the TK-CAT vector pBLCAT2 \cite{21}. Cells were split 1:3 and 24 h later transfected with 10 μg supercoiled plasmid DNA by the DEAE-dextran technique. Cells were serum starved for 16 h, after which the cells were either stimulated with IL-1β (50 pg/ml) or left untreated for 12 h and subsequently harvested for CAT assay as described previously \cite{22}.

2.2. Mobility shift assay and supershift

Gel shift assay was performed with short STAT consensus binding element (underlined) from the interferon response factor 1 (IRF-1) promoter, top strand 5’-GCC TGA TTG CCC CGA AAT GAC GGC-3’, c-fos promoter, high affinity SIS inducible element (m67SIE), top strand 5’-CAT TCC CGG TAA ATC ATG-3’ and rat β-casein promoters top strand 5’-GGG CTT CTT GAT GTA ATG GGA G-3’, end-labelled with [γ-32P]ATP (Amersham, Little Chalfont, UK). Supershift experiments were performed with anti-STAT-1 (C-136X), 0.1 (C-20X), 0.5a (L-20X), 0.5b (C-17X) (Santa Cruz, CA, USA) and anti-STAT-1 N-terminal (G16920), anti-STAT-3 N-terminal (S21320), STAT-2 (S21220), and STAT-6 (S25420) (Transduction Laboratory Inc., San Diego, CA, USA).
Labs, Lexington, KY, USA) by pre-incubation of nuclear extracts for 15 min at room temperature, or 30 min on ice before addition of probe. Nuclear extracts were prepared as described previously [23].

2.3. Immunoprecipitation and Western blots

The cells were lysed and the lysate rocked for 30 min at 4°C. For phospho (Y705) STAT-3, the lysate was cleared by adding 30 μl protein G-Sepharose (Pharmacia) for 1 h, spun down and the supernatant incubated overnight at 4°C with 1 μg/condition of a rabbit polyclonal anti-phospho (Y705) STAT-3 antibody (New England Biolabs). Goat anti-rabbit IgG (Transduction Labs) was then incubated for 30 min and the complexes pulled down with protein G-Sepharose beads after a 30 min rock. Immunoprecipitates were then washed and loaded on 10% SDS-PAGE system. The blots were probed with either a mouse anti-IL-1RαCP antibody (Transduction Labs) or STAT-3 N-terminal-specific antibodies (Transduction Labs) and detected with an anti-mouse horseradish peroxidase-conjugated secondary antibody according to the manufacturer’s instructions with an ECL kit (Amer sham). Western blot analysis was performed as described previously [23].

3. Results and discussion

IL-1β (50 pg/ml) induced DNA binding activity to the GAS element from the IRF-1 promoter (Fig. 1) which was detectable by 5 min. Binding of the STAT factor reached a maximum at 15–20 min and returned to basal within 30 min consistent with the kinetics of rapid STAT factor activation. IL-1β induced STAT DNA binding activity in nuclear extracts of the clonal insulin-secreting cell line RINm5F at concentrations that are within the exposure range that promotes islet β-cell damage [9–14,18,19]. Thus, the induction of this single DNA binding complex was observed over the concentration range of 0.5–500 pg/ml IL-1β with saturation of binding at 5 pg/ml during a 15 min incubation with the IRF-1 probe (Fig. 1). Using the IRF-1 probe, we were unable to supershift the complex with C-terminal antibodies to STAT-1, -2, -3, -5a, -5b or -6. We also found that IL-1β (50 pg/ml) induces binding to the DNA m67SIE STAT element from the c-fos promoter (Fig. 2). The STAT m67SIE complex was not supershifted with C-terminal STAT-3 antibody nor was there any induction of DNA binding activity to α-casein oligonucleotide probe in RINm5F nuclear extracts implying that STAT-5 is not a target for IL-1β (data not shown).

Nuclear extracts from 50 pg/ml IL-1β-treated RINm5F cells were also subjected to Western blot and probed with an antibody to the tyrosine phosphorylated form of STAT-3 (anti-phospho (Y705) STAT-3) and an N-terminal-directed STAT-3 antibody. This revealed that IL-1β induces tyrosine phosphor-
Fig. 4. IL-1β induces transcriptional activation from the IRE-CAT. RINm5F cells were transiently transfected with reporter plasmids driven by the STAT consensus promoter element indicated below the axis. After a period of serum starvation, the RINm5F cells were exposed to IL-1β (50 pg/ml) for 12 h and then assessed for induction of CAT activity. The IL-1α-CAT reporter construct was strongly induced whereas a slight induction at the GAS-CAT and no induction from a control plasmid pBL or βCAS-CAT constructs were observed.

![Image 99x639 to 225x778](Image 99x639 to 225x778)

Fig. 5. IL-1β induces the interaction of activated STAT-3 with the IL-1RAcP. RINm5F cells were treated with IL-1β (50 pg/ml) for the indicated time and then lysed. Immunoprecipitation was performed on whole cell lysates with an anti-phospho STAT-3 Y705 antibody. Identical immunoprecipitates were then probed with either an N-terminal directed STAT-3 antibody or an anti-IL-1RAcP monoclonal antibody on separate gels. IL-1β treatment increases the association with a peak at 2 min that rapidly declines by 10 min.

![Image 343x648 to 509x777](Image 343x648 to 509x777)

Fig. 6. Increasing intracellular cAMP attenuates the IL-1β-induced effects of IL-1β on the β-cell [18,19]. We tested whether the STAT activation mediated by IL-1β, presented in the current study, could be modulated by conditions that mimic increased metabolic activity using agents that increase intracellular cyclic adenosine monophosphate (cAMP) concentrations as occurs in normal pancreatic β-cells upon exposure to hormones that potentiate insulin secretion. GLP-1 (10 nM), an endogenously secreted peptide hormone that increases intracellular cAMP concentrations and stimulates insulin secretion in RINm5F [26] cells, caused a reduction in IL-1β (50 pg/ml)-mediated STAT-3 DNA binding (Fig. 6). Increasing the intracellular cAMP concentration with the adenylyl cyclase activator, forskolin (10 μM) and cAMP phosphodiesterase inhibitor usually mediated through STAT SH2 domain binding to tyrosine phosphorylated motifs on the receptor [17] and direct interaction of STATs with JAK are also known to occur [25]. Our data indicate that a further level of association between activated STAT-3 and the IL-1RAcP may exist. The association could be a consequence of the interaction of other regions of the STAT-3 with the IL-1RAcP complex.

High IL-1β concentrations, prolonged duration of exposure and high glucose concentrations exacerbate the deleterious effects of IL-1β on the β-cell [18,19]. We tested whether the STAT activation mediated by IL-1β, presented in the current study, could be modulated by conditions that mimic increased metabolic activity using agents that increase intracellular cyclic adenosine monophosphate (cAMP) concentrations as occurs in normal pancreatic β-cells upon exposure to hormones that potentiate insulin secretion. GLP-1 (10 nM), an endogenously secreted peptide hormone that increases intracellular cAMP concentrations and stimulates insulin secretion in RINm5F [26] cells, caused a reduction in IL-1β (50 pg/ml)-mediated STAT-3 DNA binding (Fig. 6). Increasing the intracellular cAMP concentration with the adenylyl cyclase activator, forskolin (10 μM) and cAMP phosphodiesterase inhibitor

Febs 21368 4-1-99
IBMX (50 μM) had a more pronounced effect. This may implicate a role for cAMP-dependent protein kinases in modulating IL-1β-mediated signaling. We do not know whether the STAT-3 mechanism represents the initiation of an early protective cellular mechanism or contributes to the cellular toxicity associated with inappropriate exposure to IL-1β. Our data indicate that acute elevation of β-cell metabolic activity could counteract IL-1β-mediated STAT signaling.

The events after IL-1β binding to its receptor have only recently begun to be elucidated [1–8]. Activation of the JAK/STAT pathway is associated with growing numbers of ligands [15–17]. To date, however, only one study has described a transfection of the IL-1β signaling pathway and a STAT-1-like mechanism [27]. We probed nuclear extracts of IL-1β-treated RINm5F with antibodies to STAT-1 N-terminus and phospho (Y701) STAT-1 and found that this complex is not induced in this cell line under the conditions used (data not shown). The STAT-3 isoform activation profile is somewhat different from the characterised short STAT-3 isoform. STAT-3β is a splice variant [22], which is functionally distinct from the full-length STAT-3α. Thus, STAT-3β has a higher DNA binding affinity but lacks the C-terminal transactivation domain that is believed to convey transcriptional activity to STAT-3α [28]. Indeed, STAT-3β can mediate dominant negative repression of transcription [22] or transcriptional activation [29] depending on the system studied. Thus, Sasse et al. [29] have shown that carboxy-terminal truncated STAT-3 proteins are unable to activate promoters in COS-7 cells, whilst in hepatoma cells (HepG2) they potently activate promoters and to the same extent as a full-length STAT-3. This would suggest that different mechanisms exist in different cell types to promote transcription of STAT-3 that is not always dependent on the presence of the carboxy-terminal transactivation domain. The transactivation observed in response to IL-1β in the RINm5F cells implies that the short STAT-3 isoform described here has transcriptional activity in insulin-producing cells. Regarding whether the STAT-3 mechanism mediates a protective or destructive effect, it is of note that IRF-1 is known to have a crucial role in the initiation of apoptosis and cellular growth retardation [30]. Furthermore, co-operative interaction between STAT-3β and c-Jun [31], another IL-1β-responsive transcription factor that has been linked to apoptosis [32], has also been established. However, since STAT-3 activation has been associated with diverse and often opposing phenomena, such as transformation [33] or apoptosis [32], depending on the system studied, the role of this signaling mechanism in the fate of the β-cell during type 1 diabetes will require further studies.

References