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STAT1 is Constitutively Activated in the T/C28a2 Immortalized Juvenile Human Chondrocyte Line and Stimulated by IL-6 Plus Soluble IL-6R

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Abstract

T/C28a2 immortalized juvenile human chondrocytes were employed to determine the extent to which activation of Signal Transducers and Activators of Transcription-1 (STAT1) occurred in response to recombinant human interleukin-6 (rhIL-6) or rhIL-6 in combination with the soluble IL-6 receptor (sIL-6R). Two forms of STAT1, STAT1A and STAT1B, were identified on SDS-PAGE and western blotting with anti-STAT1 antibody. Western blotting revealed that STAT1 was constitutively phosphorylated (p-STAT1). Although incubation of T/C28a2 chondrocytes with rhIL-6 (50 ng/ml) increased p-STAT1A by Δ=22.3% after 30 min, this percent difference failed to reach significance by Chi-square analysis. Similarly, no effect of rhIL-6 (Δ=+10.7%) on p-STAT1B was seen at 30 min. In contrast, although the combination of rhIL-6 plus sIL-6R had no effect on p-STAT1A, rhIL-6 plus sIL-6R increased p-STAT1B by Δ=73.3% ($p<0.0001$) after 30 min compared to the control group and by Δ=56.7% ($p<0.0001$) compared to rhIL-6 alone. Janex-1, a Janus kinase-3-specific inhibitor (100 μM) partially reduced the effect of rhIL-6 on p-STAT1B by Δ=27.7% ($p<0.05$). The results of this study showed that STAT1A/STAT1B was constitutively activated in T/C28a2 chondrocytes. Although rhIL-6 increased p-STAT1B to a small extent, the combination of rhIL-6 plus sIL-6R was far more effective in stimulating STAT1B phosphorylation compared to controls or rhIL-6 alone. These data support the likelihood that although JAK3-mediated activation of STAT1 in T/C28a2 chondrocytes may involve the IL-6/IL-6R/gp130 pathway, these results indicated that STAT1 activation in response to IL-6 preferentially involved IL-6 *trans*-signaling via sIL-6R.

Keywords: Chondrocyte; Rheumatoid arthritis; Cytokine

Introduction

Mammalian signal transducers and activators of transcription (STAT) are cytoplasmic proteins comprised of Src homology-2 regions which are found in 7 forms, STAT1, 2, 3 and 4, STAT5a, STAT5b, and STAT6 [1-3]. STAT proteins are phosphorylated by one of several activated Janus kinases, JAK1, 2, 3, 5A, 5B, 6 following cytokine and/or growth factor binding to cytokine-specific receptors [2-5]. Phosphorylated STAT proteins (p-STATs) form STAT-homodimers and/or STAT-heterodimers in the cytoplasm where they undergo nuclear translocation. In the nucleus p-STAT proteins are potent transcription factors through their ability to bind to STAT-responsive DNA sequences in gene promoter regions [6]. Under certain conditions, un-phosphorylated STATs (U-STATs) may also possess transcription factor activity [7-9].

STAT-responsive DNA motifs regulate the expression of many pro-inflammatory and anti-inflammatory cytokine genes, including interleukin-6 (IL-6), oncostatin M, IL-10, interferon-γ (INF-γ), and tumor necrosis factor-α, as well as cytokine receptor genes, such as IL-2Rα and IL-18R1 [6]. These genes have been shown to modulate the transcription of several molecules known to be critical to cell cycle progression (e.g. Fos, Cyclin-D, p21waf1, CDC25A, c-Myc, PIM1) [10], cell survival (e.g. Bcl-2, Bcl-XL, β2-macroglobulin) [11] as well as hematopoietic stem cell [12] and T-cell development [13]. Innate [14] and adaptive immune responses [15] are also regulated by activated STAT proteins.

Recently, the JAK/STAT signaling pathway was identified as a potential target for pharmacologic intervention for rheumatoid arthritis (RA) mainly because several cytokines and growth factors known to activate JAK/STAT were found at significantly elevated levels in the sera and synovial fluid of RA patients [16-19]. Based on the results from these studies, the growth factors and pro-inflammatory cytokines that appear to be most relevant to the pathogenesis and progression of RA which also activate JAK/STAT, include fibroblast growth factor [20], platelet-derived growth factor [21], INF [22], IL-7 [23], IL-17 [24], IL-23 [25], and most prominently, members of the IL-6 cytokine family, including, IL-6, oncostatin M, ciliary neutrotrophic factor, leukemia inhibitory factor, cardiotrophin-1 and adiponectin [26-31].

In general, the IL-6-type cytokines phosphorylate the identical group of JAK-receptor complexes. However, in the case of IL-6, STAT1 or STAT3 were preferentially activated through the classical or "canonical" IL-6/IL-6R/gp130-receptor-mediator signaling [3,6,32,33] (Figure 1). However, there is less evidence for STAT1 activation occurring via the interaction of IL-6 with membrane-bound IL-6R (mIL-6R) or the "non-canonical" *trans*-signaling pathway involving the interaction between IL-6 and the soluble IL-6R (sIL-6R) (Figure 1).