STAT3 Acts as a Co-activator of Glucocorticoid Receptor Signaling

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Interleukin-6 (IL-6) and glucocorticoids are important mediators of inflammatory and immunological responses. Glucocorticoids are known to synergistically enhance IL-6-mediated cellular responses. We now show that IL-6 also has a synergistic effect upon glucocorticoid signaling. In particular, IL-6-activated STAT3 associates with ligand-bound glucocorticoid receptor to form a transactivating/signaling complex, which can function through either an IL-6-responsive element or a glucocorticoid-responsive element. These findings reveal a new level of interaction between these two crucial signaling cascades and indicate that activated STAT3 can also act as a transcriptional co-activator without direct association with its DNA binding motif.

The control of gene expression by biological mediators is tightly regulated through activation of distinct transcription factors. With respect to IL-6, 1 key transcriptional events are controlled through activation of transcription factor STAT3 (1–3). Activated STAT3 forms a homodimer that translocates to the nucleus, where it enhances transcription through association with a highly conserved palindromic DNA binding motif (TTC(N)CAA) (1–5). Recent publications have shown that STAT3 also participates in the signaling events elicited by other cytokines and growth factors, including IL-10, IL-11, epidermal growth factor, growth hormone, platelet-derived growth factor, and leptin (1, 6–10).

Glucocorticoids are important mediators of the immune system and modulate the biological activities of inflammatory cytokines, such as IL-6 (11–14). Following binding to the latent intracellular glucocorticoid receptors (GR), the ligand-receptor complex translocates to the nucleus, where it activates transcription of downstream genes through interaction with the glucocorticoid-responsive element (GRE) (15–17). Recent studies have shown that the GR also regulates other signaling events through interaction with either transcription factors, such as AP-1 (Jun and Fos) (18–20), NF-kB (p65) (21, 22), and STAT5 (23), or nuclear co-factors, such as NCoR (24), CBP/p300, and pCAP (25, 26). These findings demonstrate that transcription factors also participate in signaling through direct protein/protein interaction with other transcriptional regulators (27).

It has long been recognized that glucocorticoids synergize the IL-6 response (11, 12, 28, 29). Examination of the synergistic relationship between glucocorticoid and IL-6 signaling identified a novel function for STAT3. We show that IL-6-activated STAT3 interacts with ligand-bound GR to augment glucocorticoid signaling without association with a STAT DNA binding motif. In this context, STAT3 is a potent co-activator of the glucocorticoid receptor.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Rat hepatoma H4IIE cells or COS-7 cells were cultured in minimal essential medium containing 10% fetal bovine serum (charcoal-adsorbed to remove glucocorticoids) (30). Transfection experiments were performed using the Lipofectin reagent (Life Technologies, Inc.) or calcium phosphate precipitation (31). All transfections were performed in 100-mm culture dishes using 40% confluent cells. After 16 h of transfection, the cells were trypsinized and split into 12-well plates for further treatment. 12 h later, cells were stimulated for an additional 16 h. Recombinant mouse IL-6 was used at 100 ng/ml, and dexamethasone was used at 10^{-6} M. For co-transfection assays, 5 μg of the reporter constructs were mixed with 5 μg of pβAct-GRwt (32), 2.5 μg of pCMV/IL-6ΔR, 2.5 μg of pCMV/p300, and 2.5 μg of pCMV/STAT3. An empty plasmid (pRC/CMV) was used to normalize the total amount of DNA in each transfection. For each transfection, 1 μg of pRSV-βGal vector (Promega) was always included to standardize transfection efficiency.

Luciferase and β-Galactosidase Assays—Cell lysates were prepared (33), and luciferase and β-galactosidase activities were measured using appropriate detection kits (Promega).

Co-immunoprecipitation Assays—H4IIE cells were stimulated with IL-6 (100 ng/ml) or dexamethasone (10^{-6} M) for 1 h. Nuclear extracts were prepared as described previously (33). Nuclear extracts were pre-cleared with protein A and protein G-agarose in HEGN050 buffer (10 mM HEPES (pH 8.0), 1 mM EDTA (pH 8.0), 10% glycerol, 50 mM NaCl) containing 0.1% Triton X-100 (34) and then immunoprecipitated with either 1 μg of monoclonal anti-glucocorticoid receptor antibody (BuGR2) or 1 μg of monoclonal anti-STAT3 antibody (S21320, Transduction Laboratories). Antigen-antibody complexes were isolated using protein A and protein G-agarose and washed four times in HEGN050 buffer containing 0.1% Triton X-100 (34). Protein samples were denatured, separated by SDS-polyacrylamide gel electrophoresis, and electroblotted onto polyvinylidene fluoride (PVDF) membrane for Western blot analyses with either S21320 or BuGR2 (35).

EMSA and Supershift—EMSA were performed as described previously (33, 36). The DNA probe (α2-macroglobulin) used for EMSA (5'-GATCCTCTCTGGGAATTCACA-3') is derived from the IL-6RE of the α2-macroglobulin gene promoter (36, 37). For supershift assays, nuclear extracts were preincubated for 1 h at 4 °C with 4 μg of control antibody (anti-fibrinogen), BuGR2 (monoclonal anti-GR antibody), or polyclonal anti-STAT3 antibody (C20, Santa Cruz) followed by standard EMSA procedures.

RESULTS AND DISCUSSION

The rat hepatoma cell line H4IIE possesses functional IL-6 and glucocorticoid signaling pathways (33, 38). We transiently transfected these cells with two distinct luciferase reporter constructs to elucidate the mechanism of synergy between IL-6 and glucocorticoids. The first construct (Fib-Luc) contains the proximal promoter region of the rat γ-fibrinogen gene (−300 to +54 base pairs), which has three IL-6-responsive elements (IL-6RE) but no glucocorticoid-responsive element (GRE) (33).
As expected, IL-6, but not dexamethasone, enhanced luciferase activity in these transfected cells. However, when IL-6 and dexamethasone were added together, the response was synergistic (Fig. 1A). The IL-6/dexamethasone synergy observed on the Fib-Luc construct is dependent on the integrity of all three IL-6REs, since mutation of any of these sites affects the synergistic response (33). The MMTV-Luc construct contains the mouse mammary tumor virus long terminal repeat, which has four GRE sites (23), but no IL-6RE. Although luciferase activity was not enhanced by IL-6, a synergistic response was again measured when cells were co-stimulated with IL-6 and dexamethasone (Fig. 1B). These data demonstrate that the IL-6/ glucocorticoid synergism can occur via either an IL-6RE- or GRE-containing promoter.

It has been proposed that synergy between IL-6 and glucocorticoids in hepatoma cells occurs through glucocorticoid-enhanced expression of IL-6 signaling components, namely the IL-6 receptor and gp130 (28, 29). However, this does not explain the synergy observed using the MMTV-Luc construct (Fig. 1B). To determine the importance of the IL-6 signaling components in IL-6/glucocorticoid synergism, we utilized COS-7 cells to perform reconstitution experiments. COS-7 cells do not respond to either IL-6 or glucocorticoids because expression of the IL-6 receptor, gp130, STAT3, and the glucocorticoid receptor (GR) is extremely low in these cells (3, 23, 39). The glucocorticoid (23) and IL-6 signaling pathways can be restored in COS-7 cells through the introduction of expression vectors encoding for the appropriate signaling components, the IL-6 receptor, gp130, STAT3, and the GR (Fig. 2). Co-transfection with the Fib-Luc reporter construct in this context confirms restoration of the IL-6 response (Fig. 2A). The simultaneous introduction of the GR with IL-6 signaling components resulted in an enhanced responsiveness to IL-6 and dexamethasone. Experiments using the MMTV-Luc construct (Fig. 2B) showed that dexamethasone signaling could be re-established in COS-7 cells through expression of the GR. Transfection with the IL-6 receptor, gp130, and STAT3 had no effect on MMTV-Luc luciferase activity; however, upon co-transfection with the GR, a synergistic response was observed after stimulation with IL-6 and dexamethasone. It should be pointed out that transfected cells possessing all the components but devoid of STAT3 showed no synergism. This result clearly defines the central role of STAT3 in synergistically enhancing glucocorticoid signaling. The binding of STAT3 to a conserved TCTCTGGGAA motif is the classic mechanism for STAT3 action (2, 6, 37). However, our observations suggest that STAT3 can modulate GR signaling independent of IL-6RE binding.

Ligand-bound GR can directly activate target gene transcription through the GRE (17). Furthermore, this receptor-ligand complex can modulate gene expression independently of DNA binding by associating with other transcription factors or nuclear co-factors (17, 19, 20, 23–26). We postulated that STAT3 might interact directly with the GR, thereby enhancing the transactivation of each component through its responsive element. Co-immunoprecipitation experiments were performed using nuclear extracts obtained from H4IE cells (Fig. 3). Proteins were immunoprecipitated with an anti-GR antibody and visualized by Western blotting using a STAT3 specific antibody (Fig. 3A). A STAT3-GR complex was detected only in extracts obtained from cells co-stimulated with IL-6 and dexamethasone. The STAT3-GR complex was also detected when the anti-STAT3 antibody was used for immunoprecipitation and the anti-GR antibody for Western blot analysis (Fig. 3B). A possible functional role for this complex was demonstrated by EMSA using an oligonucleotide containing a high affinity STAT3 binding site (37). IL-6 stimulation of H4IE cells induced activation of STAT3 (Fig. 3C, lanes 1–4). The inclusion of either an anti-GR antibody (lane 6) or anti-STAT3 (lane 7) in the EMSA reaction affected the protein-DNA complex. In particular, anti-GR antibodies blocked complex formation (lane 6), whereas antibodies to STAT3 supershifted the complex (lane 7). The inclusion of an equivalent amount of control antibody had no effect (lanes 5). These data indicate the presence of both the GR and STAT3 in a protein-DNA complex. Activated STAT3 is, therefore, able to bind the IL-6RE while associated with ligand-bound GR. Experimental attempts to show the STAT3-GR complex bound to a GRE probe were unsuccessful because of the weak binding of the glucocorticoid-induced complex to the GRE probe (data not shown).

It is known that GR interacts with certain transcription factors (17). The interaction between the GR and AP-1 prevents their binding to either the AP-1 site or the GRE site and antagonizes the transactivational capacities of both regulators.
STAT3 is a transcriptional co-activator.

The interaction between the GR and STAT5 enhances STAT5-mediated transactivation but diminishes GR function at the GRE site (23). Our results suggest a third type of interaction, which leads to the synergistic transactivation at both the IL-6RE and the GRE. We have demonstrated the formation of a STAT3-GR complex which can bind to the IL-6RE. In this case, GR can act as a co-activator. However, we cannot fully reconstitute the synergism using the Fib-Luc construct in COS-7 cells (Fig. 2A), suggesting that the STAT3-GR complex is insufficient for the strong synergy observed through the IL-6RE (Fig. 1A); additional factors may be required. On the other hand, we have shown that STAT3 is central to the synergy activated through the GRE (Figs. 1B and 2B). In this case, STAT3 is a potent co-activator of GRE-mediated transcription, independent of IL-6RE binding. These findings reveal a novel level of interaction between the IL-6 and glucocorticoid signaling pathways and suggest that STAT3 may modulate other glucocorticoid actions. Furthermore, since STAT3 and the GR have important roles in multiple cellular processes, such as cell growth and differentiation, cell cycle control, apoptosis, and development (32, 40–46), and since STAT3 is activated by numerous mediators other than IL-6, such as platelet-derived growth factor, epidermal growth factor, growth hormone, and leptin (10, 47), the observed interaction between STAT3 and the GR may have broad biological implications.

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