Prolactin-induced production of cytokines in macrophages *in vitro* involves JAK/STAT and JNK MAPK pathways

Anurag Tripathi and Ajit Sodhi

School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi 221005, India

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**Abstract**

Macrophages play a crucial role in host immunosurveillance against pathogens and malignancies. The enhanced productions of pro-inflammatory cytokines are central to the regulatory role of macrophages and induction of robust immune response. The excessive inflammatory response of macrophages can result into pathological conditions in host. We have previously reported that prolactin (PRL) induces the production of nitric oxide (NO) and tumor necrosis factor (TNF)-α in murine peritoneal macrophages. It was suggested that protein tyrosine kinases (PTKs), mitogen-activated protein kinases (MAPKs) and Ca<sup>2+</sup> signaling were involved in the NO production by macrophages on PRL treatment. In this manuscript, we investigated the role of PTKs [Janus kinase (JAK) 2 and phosphoinositide 3-kinase (PI3K)] and c-Jun N-terminal kinase (JNK) MAPK in PRL-induced activation of murine peritoneal macrophages. It is reported that PRL-induced activation of macrophages *in vitro* is dependent on JAK/signal transducers and activators of transcription (STAT) and JNK MAPK-signaling pathways. It is observed that pre-treatment of macrophages with JNK inhibitor, SP600125; tyrosine kinase inhibitor, genistein; PI3K inhibitor, Wortmannin and JAK2 inhibitor, AG490 inhibited the phosphorylation of JNK MAPK. Further, pre-treatment of macrophages with SP600125 inhibited the PRL-induced production of IFN-γ and TNF-α. AG490, inhibitor of JAK2, down-regulated transcription factors c-jun and STAT1 and inhibited the PRL-induced IFN-γ, TNF-α, IL-1β and IL-12p40 production in macrophages.

**Introduction**

Prolactin (PRL) has more actions than all other pituitary hormones combined (1). PRL was initially acknowledged as a neuroendocrine hormone of pituitary origin. Since 1930s, the role for PRL and other pituitary hormones in modulating the immune system has been documented (2). Clinical, animal and *in vitro* studies have shown that PRL has immunostimulatory properties (3). Apart from pituitary, many extrapituitary sites of PRL synthesis have been documented. PRL is known to be produced by T and B cells which underscore the autocrine and paracrine effects of PRL in immune system (4-6) and exhibit cytokine-like activity in human mononuclear and polymorphonuclear leukocytes (7). Further, macrophages have been reported to produce PRL and express high levels of prolactin receptor (PRLR) (8, 9).

One of the earliest biochemical events in the signaling pathway of PRL stimulation is the rapid and transient phosphorylation of specific cellular proteins including Janus kinases (JAKs) and PRLR (10, 11). JAK/signal transducers and activators of transcription (STAT)-signaling pathway has been reported to be involved not only in the immune response of numerous cytokines but also in the actions of primarily non-immune mediators such as growth factors and hormones (12, 13). Activated protein tyrosine kinases (PTKs), JAKs phosphorylate the cytoplasmic domain of the receptor, thereby creating recruiting sites for signaling proteins like STAT; STATs are phosphorylated by JAKs, dimerize and translocate to the nucleus where they regulate gene expression (14). STAT family proteins play essential role in cytokine-mediated biological responses (15). STAT1, STAT3 and STAT5 proteins have been reported to be common mediators of PRL signaling in cells of lymphoid, myeloid and mammary epithelial origin (16). PRL brings about specific activation of p91/STAT-1 in rat pre-lymphoma Nb2 cells (17). STAT3 knockout mice are highly vulnerable to endotoxin shock and macrophages from these mice atypically produce enhanced amounts of pro-inflammatory cytokines.

Mitogen-activated protein kinases (MAPKs) are essential intracellular signaling networks that eukaryotic cells use to transduce signals triggered by a wide spectrum of extracellular stimuli. They were initially discovered as low molecular weight protein kinases that were rapidly activated after stimulation with a variety of mitogens. In recent years, this family of serine/threonine kinases has been found to be activated by a number of cellular stresses also. All MAPKs are proline-directed serine/threonine protein kinases that are activated by the phosphorylation of both a threonine and a tyrosine residue in Thr-X-Tyr motif in the activation loop that lies in close proximity to the ATP- and substrate-binding sites (20). c-Jun N-terminal kinase (JNK) family members phosphorylate serine residue 63 and 73 in the N-terminal of c-jun (21), an immediate early gene product that is a member of the AP1 transcription factor complex (22). JNKs have also been described as stress-activated protein kinases since they were stimulated by a variety of cellular stresses including UV light, antioxidants, reactive oxygen species, heat, hyperosmotic shock, protein synthesis inhibitors and pro-inflammatory cytokines [IL-1 and tumor necrosis factor (TNF)] (23-29). The functional consequences of activation of JNK MAPKs are complex and still being elucidated.

Macrophages constitute the major group of phagocytic leukocytes that play a crucial role in host immunosurveillance against invading pathogens and malignancies (30-32). Activated macrophages can act as accessory cells, antigen-presenting cells and sources of cytokines for the activation of other immune effector cells (33, 34). The major cytokines produced by macrophages are pro-inflammatory like IL-1β, TNF-α, IFN-γ and IL-12 and are central to their regulatory role or in the orchestration of a robust immune response by macrophages (35, 36). In addition to the secretion of cytotoxic cytokines (IL-1 and TNF-α), activated macrophages also produce reactive oxygen and nitrogen intermediates (37, 38). The cytotoxicity of macrophages against tumor cells is well established (39). Activated macrophages can destroy tumor cells by direct cell-to-cell contact or by secretion of effector molecules like reactive nitrogen intermediates and TNF-α (40-42). PRL has been shown to enhance the cytotoxicity of NK cells against tumor cells in vivo and in vitro (43, 44).

The appropriate balance of effector molecules produced following infection and phagocytosis is important in order to clear the infection and to avoid triggering excessive inflammatory response, which may lead to pathological conditions in host (45).

We have previously reported enhanced production of nitric oxide (NO) and TNF-α in macrophages on PRL treatment and the involvement of MAPK, Ca2+ and PTKs (46). It is also observed that PRL induced enhanced production of TNF-α, IFN-γ, IL-1β and IL-12 by macrophages on treatment with PRL (A. Sochi and A. Tripathi, unpublished data). The present study was designed to elucidate the signaling mechanism involved in PRL-induced production of TNF-α, IFN-γ, IL-1β and IL-12p40 with particular reference to JAK/STAT and JNK pathways in macrophages in vitro.

Methods

Mice

Inbred strains of Balb/c mice of either sex at 8-10 weeks of age were used for obtaining peritoneal macrophages.

Cell cultures and reagents

Macrophage monolayers were cultured in RPMI 1640 medium supplemented with heat-inactivated FCS (10%), penicillin (100 U ml⁻¹), streptomycin (100 U ml⁻¹) and gentamycin (20 µg ml⁻¹) at 37°C in humidified air containing 5% CO₂. Medium RPMI 1640, TRI-reagent, luteotropic hormone or PRL (from sheep pituitary glands), pertussis toxin (PTX), Wortmannin and most of the other reagents were obtained from Sigma-Aldrich Chemicals, St. Louis, MO, USA. FCS was purchased from Biological Industries, Haemek, Israel. Genistein was from LC Services Inc., Woburn, MA, USA. SP600125, TMB8 hydrochloride and tyrophostin (AG490) were purchased from Calbiochem, La Jolla, CA, USA. Polyclonal antibodies against phospho-JAK2, phospho-nositide 3-kinase (PI3K), phospho-JNK, phospho-STAT1, phospho-c-jun, phospho-ικB, actin and HRP-conjugated anti-rabbit, anti-goat and anti-mouse IgGs were obtained from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA. All the reagents were endotoxin free as determined by the Limulus amebocyte lysate assay (sensitivity limit, 0.1 ng ml⁻¹).

Isolation and activation of macrophages

Macrophage monolayers were prepared as described previously (47). Peritoneal exudate cells were harvested from peritoneal cavity using chilled serum-free RPMI 1640 medium and added to wells of 24-well tissue culture plates (Nunc, Roskilde, Denmark). After 2-h incubation at 37°C in an atmosphere of 5% CO₂ in air in a CO₂ incubator, the non-adherent cells were removed by vigorous washing (three times) with warm serum-free RPMI 1640 medium and the adherent cells (1 x 10⁶) were incubated in complete RPMI 1640 medium overnight to form macrophage monolayers. More than 95% of the adherent cell population was macrophages as determined by morphology and non-specific esterase staining.

After overnight culture, the medium was removed from the wells and macrophage monolayers (1 x 10⁶ cells per well) were treated with PRL (100 ng ml⁻¹) in complete RPMI 1640 medium. We have previously reported that PRL induces macrophage activation at an optimal dose of 100 ng ml⁻¹ (48). Untreated macrophages were used as control. In another set, the macrophage cultures were pre-treated for 1 h with PTK inhibitor, genistein (10 µg ml⁻¹); PI3K inhibitor, Wortmannin (200 nM); Gαi-protein inhibitor, PTX (100 ng ml⁻¹); an intracellular calcium immobilizing agonist, TMB8 (100 µM); JNK inhibitor, SP600125 (10 µM) or JAK2 inhibitor, tyrophostin (AG490) (25 µM), washed and further incubated with PRL (100 ng ml⁻¹) in complete RPMI 1640 medium for different time periods. The strength of dimethyl sulfoxide in the stock solution of above inhibitors was adjusted so that its effective concentration is <0.1% when used at the recommended doses.

For western blotting with phospho-specific antibodies, the macrophage monolayers were serum starved for 6 h before treating with pharmacological inhibitors and PRL.
PTK assay

PTK activity was measured using Protein Tyrosine Kinase Assay Kit (PTK-101) from Sigma-Aldrich Chemicals. Briefly, cell lysate was prepared using lysis buffer containing activated sodium vanadate solution (according to the kit instructions). Assay was performed in a 96-well microtiter plate. One hundred and twenty-five microliters of PTK substrate solution was added to each well and plate was incubated overnight at 37°C. Coating solution was removed and each well was washed with 200 μl of washing buffer. Buffer was removed and wells were dried for 2 h at 37°C. Ninety microliters of 1× tyrosine kinase buffer containing ATP was added to each well. Twenty microliters of cell lysates was added in each well. Plate was covered and incubated at room temperature for 30 min. Reaction mixture was removed and each well was washed with 200 μl of washing buffer five times. In each well, 100 μl conjugated antibody was added. Plate was covered and incubated at room temperature for 30 min. Antibody solution was removed and each well was washed with 200 μl of washing buffer five times. One hundred microliters of freshly prepared OPD substrate solution was added to each well and incubated for 7 min in dark at room temperature. One hundred microliters of 2.5 NH2SO4 was added to each well to stop the reaction. Plate was read in a microplate ELISA reader (Emax, Molecular Devices, Menlo Park, CA, USA) at 492 nm within 30 min of addition of stop solution.

Preparation of cell lysates and immunoblotting

The macrophage monolayers with or without treatment were washed with ice-cold PBS containing 1 mM Na3VO4, lysed in 50 μl of lysis buffer [20 mM Tris–HCl, pH 8, 137 mM NaCl, 10% glycerol (v/v), 1% Triton X-100 (v/v), 1 mM Na3VO4, 2 mM EDTA, 1 mM phenylmethylsulfonylfluoride and 20 μM leupeptin and 0.15 U ml−1 aprotonin] for 20 min at 4°C. The lysates were centrifuged at 15 000 × g for 15 min and the supernatants (containing Triton X-100 soluble proteins) were separated on 10% SDS–polyacrylamide gels. The separated proteins (40 μg per lane) were transferred to nitrocellulose membrane (1 h at 350 mA) using Bio-Rad Mini Transblotter and immunoblotted with primary antibody, incubated with secondary antibody conjugated with HRP and visualized by the Chemiluminescence Western Blotting Kit (Santa Cruz Biotechnology Inc.). To monitor equal loading of protein, western blot analysis using antibody directed against actin was done for each experiment as shown in lower panels.

The densitometric analysis is shown below the blots. Densitometric analysis was done using the Gene Tools software from Syngen, a Division of Synoptics Ltd, Cambridge, UK. The densities of each band are represented as Raw volume.

Assay for IL-1β, IL-12p40, IFN-γ and TNF-α

Murine peritoneal macrophages were treated with PRL for different time periods. IL-1β, IL-12p40, IFN-γ or TNF-α was measured in supernatant by commercial ELISA kits (BD PharMingen, San Diego, CA, USA).

Percentage viability by MTT assay

Percentage viability of macrophages was determined by 3(4,5) dimethyl thiazol-2,5-diphenyl tetrazolium bromide [MTT] assay as described earlier (26). The relative cell viability was calculated according to the formula:

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\text{Relative cell viability} = \frac{\text{absorbance experimental}}{\text{absorbance control}} \times 100,
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where ‘absorbance control’ represents macrophages incubated in medium alone and ‘absorbance experimental’ represents macrophages treated with PRL. PRL plus AG490, Wortmannin, TMB8, PTX, SP600125 or their vehicles. In each of the experiments, the viability of the peritoneal macrophages was not affected by the doses of the PRL, AG490, Wortmannin, TMB8, PTX, SP600125 or their vehicles used up to 48 h.

Statistical analysis

The statistical significance of difference between the test groups was analyzed by Student’s t-test (two tailed) using SPSS 7.5 software. Statistical significance was assumed at \( P < 0.05 \). The error bars of the values represent 95% confidence interval.

Results

PTK activity in PRL-treated macrophages

Macrophages were treated with PRL to check if it induces increased PTK activity, which is essential for the phosphorylation and activation of molecules involved in signal transduction.

PTK activity was measured in cell lysates of macrophages treated with PRL for various time intervals. PRL treatment significantly enhanced PTK activity in macrophages. Maximum activity was observed at 5–15 min (Fig. 1a). Pre-treatment of macrophages with pharmacological inhibitor of JAK2, AG490; PTK inhibitor, genistein or PI3K inhibitor, Wortmannin significantly inhibited the PRL-induced PTK activity (Fig. 1b).

PRL induces phosphorylation of JAK2 in macrophages

Macrophages on treatment with PRL expressed enhanced expression of phosphorylated JAK2 as observed by immunoblotting. The expression of phosphorylated JAK2 was found to increase from 2.5 to 15 min of PRL treatment and a decrease thereafter (Fig. 2a). Untreated macrophages did not show the expression of phosphorylated JAK2. To confirm the specificity of PRL-induced JAK2 phosphorylation, macrophages were pre-treated with JAK2 inhibitor, AG490 (25 μM) for 1 h, washed and then treated with PRL for 5 min. It was observed that AG490 inhibited the expression of PRL-induced phosphorylated JAK2 in a dose-dependent manner (Fig. 2b). Maximum inhibition was observed with 25 μM of AG490.

The pre-treatment of macrophages with Wortmannin, an inhibitor of PI3K, did not effect the PRL-induced expression of phosphorylated JAK2, whereas pre-treatment with AG490 abrogated the PRL-induced expression of phosphorylated JAK2 (Fig. 2c).
PRL induces phosphorylation of PI3K in macrophages

PRL treatment induced enhanced expression of phosphorylated PI3K in macrophages. The expression of phosphorylated PI3K increased from 5 to 30 min of PRL treatment and decreased thereafter (Fig. 3a).

Pre-treatment of macrophages with JAK2 inhibitor, AG490 (25 µM) significantly inhibited PRL-induced phosphorylation of PI3K (Fig. 3b).

PRL induces phosphorylation of JNK MAPK in macrophages

PRL treatment of macrophages induced phosphorylation of JNK, which was found to increase from 5 to 15 min of PRL treatment and a decrease thereafter (Fig. 4a).

To confirm the specificity of PRL-induced JNK phosphorylation, macrophages were pre-treated with JNK inhibitor,
SP600125 (10 µM) for 1 h, washed and then treated with PRL for 15 min. It is observed that SP600125 inhibited the expression of PRL-induced phosphorylated JNK in a dose-dependent manner (Fig. 4b).

Expression of phosphorylated STAT1 in PRL-treated macrophages and effect of AG490, Wortmannin and SP600125 on its expression

PRL treatment induced enhanced expression of phosphorylated STAT1 in macrophages. The expression of phosphorylated STAT1 increased from 2.5 to 15 min of PRL treatment (Fig. 5a).

Pre-treatment of macrophages with JAK2 inhibitor, AG490 (25 µM) significantly inhibited PRL (100 ng ml⁻¹)-induced phosphorylation of STAT1. PI3K inhibitor, Wortmannin (200 nM) or JNK inhibitor, SP600125 (10 µM) did not inhibit PRL-induced phosphorylation of STAT1 (Fig. 5b).

Expression of phosphorylated c-jun in PRL-treated macrophages and effect of AG490, Wortmannin and SP600125 on its expression

Macrophages on treatment with PRL showed enhanced expression of phosphorylated c-jun, which was found to increase from 30 to 90 min of PRL treatment (Fig. 6a).

Pre-treatment of macrophages with JAK2 inhibitor, AG490 (25 µM); PI3K inhibitor, Wortmannin (200 nM) or JNK inhibitor, SP600125 (10 µM) significantly inhibited PRL (100 ng ml⁻¹)-induced phosphorylation of transcription factor c-jun (Fig. 6b).

Effect of AG490, Wortmannin and SP600125 on the expression of PRL-induced phospho-IkB

Macrophages when treated with PRL expressed significantly enhanced expression of phosphorylated IkB. Pre-treatment of macrophages with JAK2 inhibitor, AG490 (25 µM) resulted in inhibition of phosphorylation of IkB, as observed by immunoblotting (Fig. 7).

The pre-treatment of macrophages with PI3K inhibitor, Wortmannin and JNK inhibitor, SP600125 did not inhibit the PRL-induced expression of phosphorylated IkB (data not shown).

Role of JNK MAPK in the production of IFN-γ, TNF-α, IL-1β and IL-12p40

Macrophages on treatment with PRL produced significantly enhanced amounts of IFN-γ, TNF-α, IL-1β and IL-12p40. Pre-treatment of macrophages with JNK inhibitor, SP600125 (10 µM) significantly inhibited PRL-induced production of IFN-γ and TNF-α, but did not affect PRL-induced IL-12p40 or IL-1β production. Macrophages treated with SP600125 alone exhibited minimal production of cytokines as in the untreated macrophages (Fig. 8).

Role of JAK2 in the production of IFN-γ, TNF-α, IL-1β and IL-12p40

Pre-treatment of macrophages with JAK2 inhibitor, AG490 (25 µM) significantly inhibited PRL-induced production of IFN-γ, TNF-α, IL-12p40 and IL-1β (Fig. 9).

Discussion

The present investigation was undertaken to study the role of JAK2, PI3K and JNK MAPK in the activation of macrophages on treatment with PRL in vitro. We have previously

Inhibition of PRL-induced phospho-JNK expression in macrophages by genistein, AG490 and Wortmannin

Pre-treatment of macrophages with PTK inhibitor, genistein (10 µg ml⁻¹); JAK2 inhibitor, AG490 (25 µM) or PI3K inhibitor, Wortmannin (200 nM) significantly inhibited PRL-induced phosphorylation of JNK MAPK (Fig. 4c).

Ga1-protein inhibitor, PTX (100 ng ml⁻¹) or intracellular calcium immobilizing agent, TMB8 (100 µM) did not have any effect on PRL-induced JNK phosphorylation.
reported the production of NO and TNF-α (48) and have also observed increased production of IL-1β, IFN-γ and IL-12p40 by macrophages (A. Sodhi and A. Tripathi, unpublished data) on treatment with PRL. The role of PTKs, Ca** and MAPKs in the induction of inducible nitric oxide synthase (iNOS) and NO production by macrophages on PRL treatment has been reported (48).

PTKs, JAKs, are constitutively associated with the PRLRs and it is likely that ligand binding stabilizes the preformed receptor JAK complex. JAK/STAT pathway has been known to mediate most of the pleiotropic effects of PRL in diverse cell types (49). We demonstrate that PRL-induced phosphorylation of JAK2 is rapid and detectable within 2.5 min of PRL treatment. Maximum expression of phospho-JAK2 occurs at 5–15 min. PRL also induced the phosphorylation of PTK, PI3K, which was detectable within 5 min and peaking at 15–30 min. Pre-treatment of macrophages with AG490, a specific inhibitor of JAK2 inhibited the phosphorylation of JAK2 at 5 min and PI3K at 30 min. Wortmannin, a specific inhibitor of PI3K did not affect the phosphorylation of JAK2. These observations suggest that PI3K acts downstream to JAK2 in the signaling cascade on treatment of macrophages with PRL.

Though JNK pathway is not very well documented in PRL signal transduction, a recent report suggests time- and dose-dependent activation of JNK MAPK in PRL-dependent Nb2 cell line (50). We have observed that PRL induces the activation of p38 MAPK in macrophages (A. Sodhi and A. Tripathi, unpublished data). Simultaneous activation of p38 and JNK MAPK is a general feature observed in most inflammatory reactions (51, 52). Therefore, the expression of phospho-JNK on PRL stimulation was also studied. Activation (phosphorylation) of JNK MAPK was observed on PRL treatment with maximum phosphorylation at 15 min of treatment. It is further observed that phosphorylation of JNK was significantly inhibited on pre-treatment of macrophages with AG490 (specific inhibitor of JAK2) and Wortmannin (specific inhibitor of PI3K). Pre-treatment of macrophages with TMB8 and PTX did not inhibit the phosphorylation of JNK, which suggests that Ca**-signaling pathway and G-proteins are not involved in the upstream signaling events leading to the activation (phosphorylation) of JNK MAPK.

Fig. 4. (a) Expression of phospho-JNK MAPK in macrophages treated with PRL (100 ng ml⁻¹) for different time periods as checked by immunoblotting. Lane 1, untreated; lane 2, PRL (5 min); lane 3, PRL (15 min); lane 4, PRL (30 min) and lane 5, PRL (60 min). The figures are representative of three independent experiments having similar results. (b) Effect of SP600125 on PRL (100 ng ml⁻¹)-induced phospho-JNK expression as checked by immunoblotting. Macrophages were pre-treated with or without various doses of SP600125 for 60 min. Thereafter, macrophages were washed, treated with PRL (100 ng ml⁻¹) in fresh medium for 15 min and expression of phospho-JNK checked by immunoblotting. Lane 1, untreated; lane 2, PRL; lane 3, PRL + SP600125 (1 μM); lane 4, PRL + SP600125 (5 μM) and lane 5, PRL + SP600125 (10 μM). The figures are representative of three independent experiments having similar results. (c) Effect of genistein (G), AG490 and Wortmannin (W) on the expression of phospho-JNK MAPK protein in PRL (100 ng ml⁻¹)-treated macrophages as checked by immunoblotting. Macrophages were pre-treated with inhibitors for 1 h. Thereafter, macrophages were washed, treated with PRL (100 ng ml⁻¹) in fresh medium for 15 min and expression of phospho-JNK checked by immunoblotting. Lane 1, untreated; lane 2, PRL; lane 3, PRL + AG490 and lane 5, PRL + W. The figures are representative of three independent experiments having similar results.
These observations suggest the involvement of JAK2/PI3K/JNK-signaling cascade in PRL-treated macrophages. Expression of transcription factors c-jun and STAT1 plays a vital role in the inflammatory responses (53). It has been reported that STAT1-deficient mice fail to induce iNOS on IFN treatment (54, 55). It is observed that PRL induces phosphorylation of STAT1 and c-jun. Pre-treatment of macrophages with SP600125, AG490 or Wortmannin resulted in the inhibition of PRL-induced phosphorylation of JNK and c-jun. However, only AG490 inhibited the phosphorylation of STAT1, suggesting STAT1 to be downstream of JAK2. Pre-treatment of macrophages with Wortmannin or SP600125 did not affect the phosphorylation of STAT1, indicating its independent regulation from PI3K- or JNK-signaling cascade.

SP600125, a specific inhibitor of JNK, significantly inhibited the production of IFN-γ and TNF-α but not of IL-1β and IL-12p40, whereas AG490 (JAK2 inhibitor) significantly inhibited the production of IFN-γ, TNF-α as well as IL-1β and IL-12p40. These observations suggest the involvement of JNK MAPK in PRL-induced production of IFN-γ and TNF-α. IL-1β and IL-12p40, therefore, may be regulated by some other signaling cascade downstream to JAK2. PRL-induced phosphorylation of IκB relates to the activation of transcription factor nuclear factor-κB (NF-κB). The expression of phosphorylated IκB is a measure of NF-κB activation. On phosphorylation, IκB dissociates itself from NF-κB–IκB complex, resulting in the translocation of NF-κB from cytoplasm to the nucleus (56). Since AG490 inhibits the PRL-induced phosphorylation of IκB, it suggests NF-κB to be a downstream mediator of JAK2 pathway.

High levels of PRL and pro-inflammatory cytokines have been found in the active phase of autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (57, 58). The aberrant production of these pro-inflammatory
cytokines could be a direct consequence of high serum PRL levels. Thus, it is essential to elucidate the signaling mechanism involved in the production of these pro-inflammatory cytokines on treatment of macrophages with PRL. SP600125, developed by Celgene, inhibits JNK2 but does not interfere with the extracellular signal-regulated kinase or p38 MAPK pathway and reduces paw swelling in a rat model of inflammatory arthritis (59). Thus, pharmacological JNK inhibition has important anti-inflammatory consequences and emerges as a promising approach for combating inflammatory disease in human pathology (60). Our studies also suggest the role of JAK2 and JNK signaling in the production of pro-inflammatory cytokines by macrophages on PRL treatment. The JAK2 and JNK inhibitors (AG490 and SP600125) could be important tools in the control of pro-inflammatory disorder especially autoimmune diseases like rheumatoid arthritis where abnormal PRL serum level may be responsible for disease pathogenesis.

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Abbreviations
iNOS inducible nitric oxide synthase
JAK Janus kinase
JNK c-Jun N-terminal kinase
MAPK mitogen-activated protein kinase
NF-κB nuclear factor-κB
NO nitric oxide
PI3K phosphoinositide 3-kinase
PTK protein tyrosine kinase
PRL prolactin
PTLR prolactin receptor
PTX pertussis toxin
STAT signal transducers and activators of transcription
TNF tumor necrosis factor

References


JAK/STAT and JNK in PRL-induced macrophage activation


