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Abstract

IL-3 induces tyrosine phosphorylation of both JAK1 and JAK2. JAK1 and JAK2 are protein tyrosine kinases involved in the regulation of cell proliferation, differentiation, and survival. These proteins may play a key role in mediating the effects of the cytokine, IL-3, on hematopoietic cells. However, it is not clear which regions of JAK1 or JAK2 are associated with IL-3 receptor. In the work reported here, we demonstrated that JAK2 and JAK1 are preassociated with the IL-3 receptor alpha and beta subunits, respectively. Furthermore, we constructed the several plasmids of JAK1 or JAK2 deletion mutants for in-vitro translation and GST pull-down assay. In the GST pull-down assay, JAK1 associates with IL-3 receptor beta subunit via the JH7–JH3 domains. Deletion of JAK2 JH7–6 domains impaired the association of IL-3 receptor alpha subunit with JAK2. Finally, whether these regions affect the IL-3 signal transduction and IL-3-dependent cell activity will be studied.

Keywords: JAK1, JAK2, IL-3 receptor, association

Introduction

The cytokine, interleukin-3 (IL-3), regulates cell proliferation, differentiation, and anti-apoptosis of hematopoietic cells.[1,2] IL-3 performs these functions through specifically binding to the IL-3 receptor α subunit (IL-3Rα) and recruiting the β subunit to form a heterodimer.[3,4] While the α subunit is specific to each cytokine, the β subunit, known as β common (βc), is shared by two other cytokines, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF). The mouse has two highly homologous β subunits, βc and IL-3Rβ (β_{IL-3}), while humans have one type of β subunit (βc). IL-3R belongs to the cytokine receptor superfamily. Although IL-3R does not possess kinase activity, it likely associates with and requires transducing signals from the cytoplasmic Janus tyrosine kinases (JAKs).[5] IL-3 has been shown to induce tyrosine phosphorylation of JAK1 and JAK2.[6-8] The JAKs (JAK1, JAK2, JAK3, and Tyk2) are a family of nonreceptor tyrosine kinases.[9] One downstream effect of JAK activation is the phosphorylation of signal transduction and activation of transcription (STAT) proteins. The phosphorylated STAT proteins then translocate into the nucleus and regulate the transcription of specific genes.[5,9] In addition to STAT, phosphatidylinositol 3-kinase (PI-3K)/Akt, and mitogen-activated protein kinase (MAPK) pathways are also activated by JAK.[10-14] STAT, Akt, and MAPK have been shown to regulate cell proliferation and anti-apoptosis activities.[15-18]

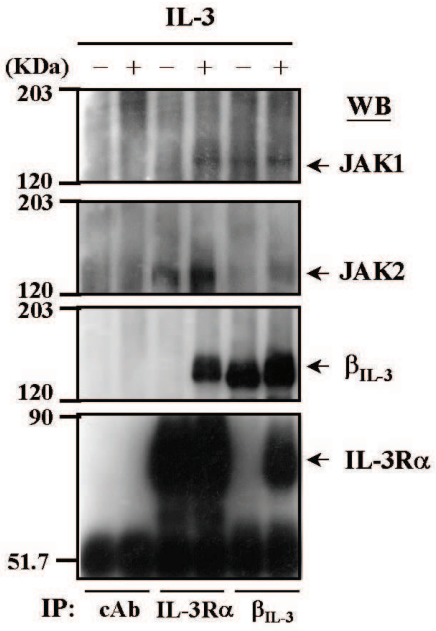
The interaction between JAK2 and single subunit cytokine receptors [*i.e.* erythropoietin receptor (EPOR), growth hormone receptor, and prolactin receptor (PRL-R)] as well as heterodimeric receptors (IFNγR and interleukin-5 receptors) has been extensively characterized.[19,20] The membrane proximal region of cytokine receptors that interact with JAK2 contains a highly conserved motif termed Box 1, which is formed by a proline-rich sequence located 6–10 amino acids after the transmembrane domain.[21,22] However, which regions of JAKs interact with IL-3 receptor are now unclear. We describe the unassociated state of IL-3Rα and β subunits in the absence of IL-3. JAK2 and JAK1 are preassociated with the IL-3 receptor alpha and beta subunits, respectively. Following IL-3 stimulation, JAK2 proteins are associated with the IL-3 receptor beta subunit and JAK1 proteins are associated with the IL-3 receptor alpha subunit. Furthermore, we identify the regions of JAK1 and JAK2 binding to IL-3 receptor.

Results

JAK2 and JAK1 interact with IL-3Rα and β_{IL-3}, respectively

Previous reports have revealed that JAK is preassociated with the cytokine receptor.[21,22] Whether JAK1 and JAK2 can also interact with the IL-3 receptor was examined here. IL-3 starved Ba/F3 cells were stimulated with or without IL-3 and cell lysates were prepared. Cell lysates were then immunoprecipitated with anti-IL-3Rα or -β_{IL-3} antibodies followed by Western blotting with anti-JAK1 and anti-JAK2 antibodies. As shown in Figure 1, JAK2 was coimmunoprecipitated with IL-3Rα whereas JAK1 was coimmunoprecipitated with β_{IL-3}, regardless of IL-3 stimulation. However, JAK2 was coimmunoprecipitated with β_{IL-3} and JAK1 was coimmunoprecipitated with IL-3Rα, only after IL-3 stimulation. These results indicate that JAK2 and JAK1 interact constitutively with IL-3Rα and β_{IL-3}, respectively.

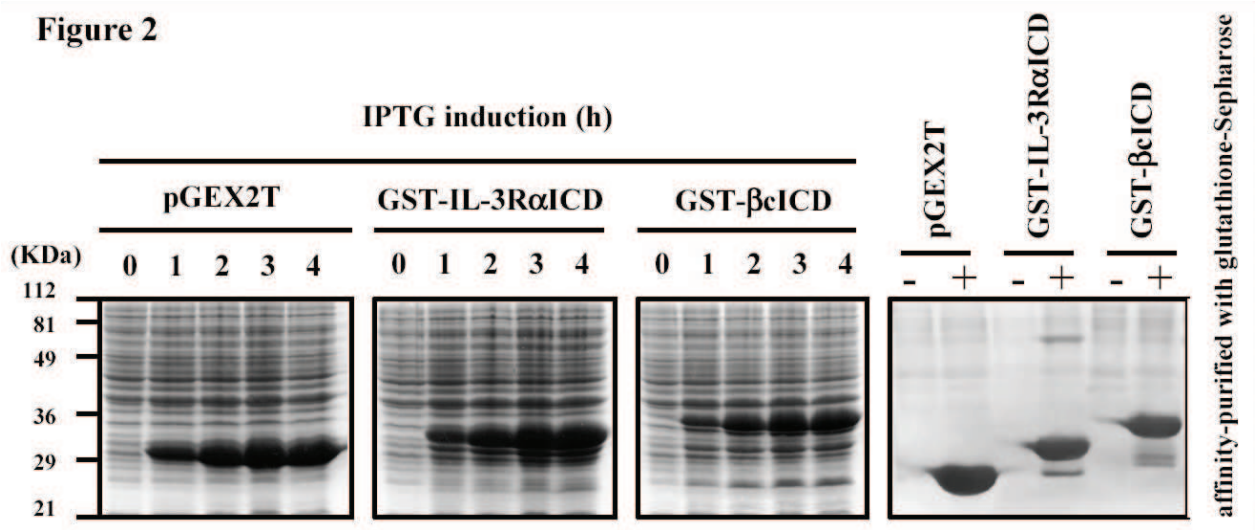
Figure 1



Constructs of glutathione S-transferase fusion proteins and JAKs deletion mutants

cDNA constructs encoding the glutathione S transferase (GST) fusion protein for the entire IL-3Rα and βc intracellular domains were obtained by inserting the polymerase chain reaction (PCR) fragments of IL-3Rα (GST-IL-3RαICD) and βc (GST-βc) into the pGEX2T vector. These GST fusion proteins were expressed in *Escherichia coli,* and were induced with IPTG for 1 to 4 hours. Furthermore, these GST fusion proteins were affinity-purified on glutathione-Sepharose

(Figure 2).

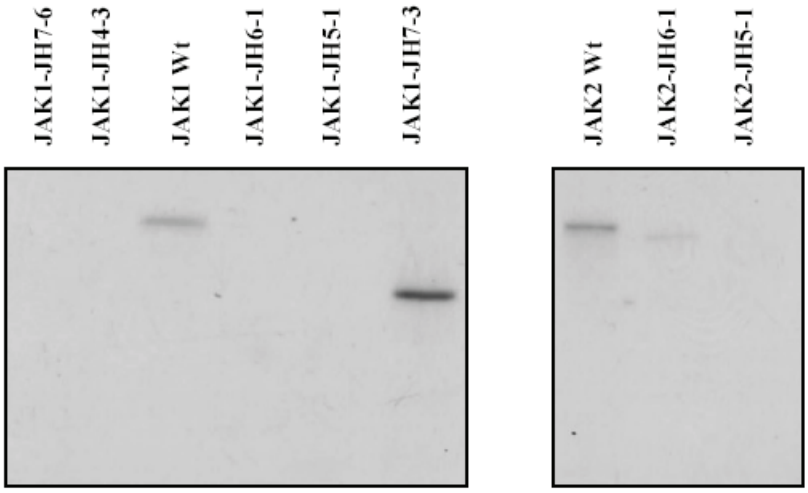


The plasmids of JAK1 and JAK deletion mutants were constructed by restricted enzymes digestion or PCR (Figure 3).

To map the regions of JAK1 and JAK2 for binding to IL-3 receptor

The interaction of GST fusion proteins to the deletion mutants of JAK1 and JAK2 were examined. Equal amounts of GST fusion proteins were incubated with lysates (input data ont shown) of in vitro transcription/translation. After adding sample buffer, the precipitates bound to GST fusion proteins were eluted by boiling and examined with autoradiograph (Figure 4). These data showed that the JH7-JH3 domains of JAK1 and the JH7-6 domains of JAK2 are essential for binding to IL-3 receptor beta and alpha subunit, respectively.

Figure 4



Discussions

In this study, In this study, we demonstrated that IL-3Rα and βc were not associated in the absence of IL-3, and that the βc would associate with IL-3Rα only after IL-3 stimulation (Figure 1), consistent with a recent report.[23] Based on our results, we propose the following mechanism for the simultaneous activation of JAK1 and JAK2 proteins by IL-3. Prior to IL-3 induction, JAK2 and JAK1 proteins exist in a preassociated state with IL-3Rα and β_{IL-3}, respectively (Figure 1). When IL-3 binds to IL-3Rα, a large functional complex of JAK2-IL-3Rα and JAK1-β_{IL-3} is formed. JAK1, JAK2, and β_{IL-3} within this functional complex undergo tyrosine phosphorylation, thereby triggering further downstream signaling that results in cell proliferation and in the suppression of apoptosis. Our proposed model is consistent with the single chain cytokine receptor (i.e., receptors for growth hormone and erythropoietin) model that predicts that the JAK2 protein is activated by homodimeric complexes of cytokine receptor-JAK2 and cytokine receptor-JAK2 after cytokine binding to its receptor.[24] The physical interaction between the IL-3 receptor and the JAKs proteins, demonstrated in our system (Figure 1), is consistent with that seen in the IL-5 system, in which JAK2 and JAK1 proteins were shown to be constitutively associated with IL-5Rα and βc, respectively.[20] Other studies have also reported ligand-induced JAK2 binding to the β subunit in the IL-3 or GM-CSF system.[25-27] Our study, however, provides an explanation for the mechanism by which IL-3 induces heterodimerization of IL-3Rα with the β_{IL-3}, enabling JAK2-IL-3Rα to interact with the β_{IL-3}. Similarly, JAK1-β_{IL-3} has the opportunity to interact with

the α subunit (Figure 1).

Recent work by Lacronique et al has revealed that fusion proteins that contain the oligomerization domain of TEL and the tyrosine kinase domain of JAK1, JAK2, JAK3 or TYK2 have similar characteristics and can effectively substitute for the survival and mitogenic signals of IL-3.[28] That is, the tyrosine kinase domain [JH1 (JAK homology) domain] from the four members of the JAK family is not specific in IL-3 signaling. In addition to the JH1 domain, the JAK family contains the JH2-JH7 domain. The sequence of the JH2 domain is similar to that of the JH1 kinase domain, except for the lack of kinase activity. JH2 has been suggested to have a negative regulatory effect on JAK2 kinase activity. Deletion of JH2 from JAK2 constitutively activated the cytokine receptor, independent of cytokine, but signal transduction activity was lower than that of wild-type JAK2 stimulated by cytokine.[29] There is much sequence variation within the N-terminal JH3-JH7 domains of the four members of the JAK family. These domains have been implicated in receptor association[30] and in controlling the kinase activity of JAK3,[31] thus implying that they could be involved in controlling JAK kinase activity and in signal transduction. Therefore, the JH2-JH7 domains may be responsible for the functional specificity of JAK, which warrants further studies on the analyses of the functions of wild-type JAK.

The GST pull down assay (Figure 4) is one time experiment, we will further confirm this data. Future studies in our laboratory will analyze whether the binding regions of JAKs (JAK1 and JAK2) to IL-3 receptor play a negative role on the initiation of IL-3 receptor signaling and/or cell proliferation.

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