Both the suppressor of cytokine signaling 1 (SOCS-1) kinase inhibitory region and SOCS-1 mimetic bind to JAK2 autophosphorylation site: implications for the development of a SOCS-1 antagonist.

Abstract:

Suppressor of cytokine signaling (SOCS)-1 protein modulates signaling by IFN-gamma by binding to the autophosphorylation site of JAK2 and by targeting bound JAK2 to the proteosome for degradation. We have developed a small tyrosine kinase inhibitor peptide (Tkip) that is a SOCS-1 mimetic. Tkip is compared in this study with the kinase inhibitory region (KIR) of SOCS-1 for JAK2 recognition, inhibition of kinase activity, and regulation of IFN-gamma-induced biological activity. Tkip and a peptide corresponding to the KIR of SOCS-1, ((53))DTHFRTFRSHSDYRRI((68)) (SOCS1-KIR), both bound similarly to the autophosphorylation site of JAK2, JAK2(1001-1013). The peptides also bound to JAK2 peptide phosphorylated at Tyr(1007), pJAK2(1001-1013). Dose-response competitions suggest that Tkip and SOCS1-KIR similarly recognize the autophosphorylation site of JAK2, but probably not precisely the same way. Although Tkip inhibited JAK2 autophosphorylation as well as IFN-gamma-induced STAT1-alpha phosphorylation, SOCS1-KIR, like SOCS-1, did not inhibit JAK2 autophosphorylation but inhibited STAT1-alpha activation. Both Tkip and SOCS1-KIR inhibited IFN-gamma activation of Raw 264.7 murine macrophages and inhibited Ag-specific splenocyte proliferation. The fact that SOCS1-KIR binds to pJAK2(1001-1013) suggests that the JAK2 peptide could function as an antagonist of SOCS-1. Thus, pJAK2(1001-1013) enhanced suboptimal IFN-gamma activity, blocked SOCS-1-induced inhibition of STAT3 phosphorylation in IL-6-treated cells, enhanced IFN-gamma activation site promoter activity, and enhanced Ag-specific proliferation. Furthermore, SOCS-1 competed with SOCS1-KIR for pJAK2(1001-1013). Thus, the KIR region of SOCS-1 binds directly to the autophosphorylation site of JAK2 and a peptide corresponding to this site can function as an antagonist of SOCS-1