Simultaneous activation of JAK1 and JAK2 confers IL-3 independent growth on Ba/F3 pro-B cells

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Abstract

JAK1 and JAK2 are 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. IL-3 induces tyrosine phosphorylation of both JAK1 and JAK2. However, it is not clear whether the activation of JAK1, JAK2, or both is sufficient to confer factor-independent growth in IL-3 dependent cells. To address this issue, fusion proteins CD16/CD7/JAK (CDJAK), comprised of a CD16 extracellular domain, a CD7 transmembrane domain, and a JAK cytoplasmic region (either a wild-type JAK or a dominant negative mutant of JAK) were constructed. We established several Ba/F3 derivatives that stably overexpress the conditionally active forms of either CDJAK1, CDJAK2, or both these fusion proteins. In this study, the autophosphorylation of CDJAK1 or CDJAK2 was induced by crosslinking with anti-CD16 antibody. We demonstrated that, like their wild-type counterparts, CDJAK1 and CDJAK2 were preassociated with the IL-3 receptor beta and alpha subunits, respectively. Furthermore, the simultaneous activation of both CDJAK1 and CDJAK2 fusion proteins, but not either one alone, led to the tyrosine phosphorylation of the IL-3 receptor beta subunit, the activation of downstream signaling molecules, including STAT5, Akt, and MAPK, and the conferring of factor-independent growth to IL-3-dependent Ba/F3 cells. Coexpression of dominant negative mutants CDJAK1KE or CDJAK2KE with wild type CDJAK2 or CDJAK1, respectively, inhibited these activation activities. These results suggest that JAK1 and JAK2 must work cooperatively and not independently and that their actions are dependent on having normal kinase activity to trigger downstream signals leading to IL-3 independent proliferation and survival of Ba/F3 cells.