Regulation of JAK3 Expression in Human Monocytes: Phosphorylation in Response to Interleukins 2, 4, and 7

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Summary

The Janus family of kinases (JAKs) has been shown to be involved in the signal transduction of a number of cytokine receptors. Recently, we have cloned a novel JAK family member, JAK3, that is expressed in natural killer and activated T cells and is coupled functionally and physically to the interleukin 2 (IL-2) receptor in these cells. Here we report that JAK3 was expressed at low but detectable levels in human monocytes. In contrast, JAK3 expression was strongly induced during activation by interferon γ (IFN-γ) or lipopolysaccharide. Moreover, JAK3 became tyrosine phosphorylated in response to IL-2, IL-4, and IL-7 but not response to IFN-γ or granulocyte/macrophage colony-stimulating factor. Together, these findings suggest that JAK3 is functionally important in activated monocytes and cells of the myeloid lineage and is involved in signaling responses of cytokines that use the common γ-chain of the IL-2 receptor.

Many of the regulatory networks that govern the maturation and function of leukocytes are mediated by cytokines that bind to receptors of the hematopoietin family (1). Although the proteins of this structurally related family do not possess intrinsic tyrosine kinase activity, tyrosyl phosphorylation is an early and requisite event in the signal transduction pathways of many of these receptors suggesting the involvement of cytoplasmic protein tyrosine kinases (2). Recently, the nonreceptor tyrosine kinases belonging to the Janus (JAK) family (JAK1, JAK2, and Tyk2) have emerged as key elements in the signaling pathway elicited by the interferons as well as several cytokines that use hematopoietin family receptors (3, 4). Binding of these cytokines to their respective receptors results in the tyrosine phosphorylation of various combinations of JAK family kinases. For example, Tyk2 and JAK1 are coupled to the receptors for IFN-α/β (5), whereas IFN-γ induces phosphorylation of JAK1 and JAK2 (6).

1 Abbreviation used in this paper: JAK, Janus family of kinases.

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Materials and Methods

Cell Culture. Monocytes were purified from PBMC by centrifugal elutriation as described elsewhere (21). The purity of the monocyte preparation used in this study was 93 ± 3% as assessed by morphology on Giemsa-stained cytospin preparations and flow cytometry using the monocyte-specific mAb LeuM3. Peripheral blood monocytes, U937, and THP-1 cells were cultured in RPMI 1640 (Advanced Biotechnologies, Inc., Columbia, MD), containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/liter l-glutamine, 20 mmol/liter Hepes (GIBCO BILL, Gaithersburg, MD), and 10% heat-inactivated fetal bovine serum (HyClone Laboratories, Logan, UT). The TF-1 (22) and M07e (Genetics Institute, Cambridge, MA) cell lines were supplemented with 10 ng/ml GM-CSF or GM-CSF plus 100 ng/ml stem cell factor (SCF), respectively. Human recombinant IFN-γ (lot N9027AX, sp act 2.02 × 10^6 IU/mg) was kindly provided by Dr. H. M. Shepard (Genentech Labs, San Francisco, CA). Human recombinant IL-4, GM-CSF, and SCF were obtained from Pepro Tech (Rocky Hill, NJ). Recombinant purified IL-2 from Escherichia coli (lot LP-381, sp act. 18 × 10^6 IU/mg, and LPS content <0.0006 ng/ml) was kindly provided by Cetus Corp. (Emeryville, CA).

Northern Blot Analysis. For RNA extraction, 5 × 10^7 monocytes were plated in 25 ml medium alone or supplemented with the indicated cytokines. Total RNA was isolated and Northern blots were performed as described (21). The cDNA probe used corresponded to the JH1 and JH2 domains of JAK3 (7). All gels were inspected visually before transfer and each filter was subsequently stripped of antibody and probed with anti-JAK3 antibody to confirm the specificity of the JAK3 antibody. These filters were then rehybridized with a probe specific for G3PDH (Clontech, Palo Alto, CA) to ensure that equal amounts of RNA were loaded in each lane.

Western Blot Analysis. For analysis of JAK3 protein levels, cells were washed in PBS and lysed in buffer containing 1% Triton X-100. Insoluble material was removed by centrifugation, the protein content was determined using a protein assay kit (Bio-Rad Laboratories, Richmond, CA), and equal amounts of protein for each sample were subjected to SDS-PAGE, then electrophoretically transferred to Immobilon (Millipore Corp., Bedford, MA) as described (7). The resulting filters were probed with a polyclonal rabbit serum raised against a synthetic peptide corresponding to the COOH-terminal region of the JAK3 protein (amino acids 1104-1124) (7). Bound rabbit antisera was detected using goat anti-rabbit horseradish peroxidase-linked antisera (Boehringer Mannheim Corp., Indianapolis, IN) detected by the enhanced chemiluminescence (ECL) method (Amersham, Arlington Heights, IL).

Results

JAK3 Expression in Myeloid Cell Lines and Its Phosphorylation by IL-2 and IL-4 in M07e. In addition to activated lymphoid cells, JAK3 has been reported to be expressed in the growth factor–dependent, murine myeloid cell line 32D but not in resting human monocytes (8, 11, 20). In 32D cells transfected with the IL-2 receptor, JAK3 was tyrosine phosphorylated upon treatment of the cells with IL-2 (11). These data prompted us to ask whether human factor–dependent myeloid cell lines with endogenous receptors for IL-2 or IL-4 might express appreciable JAK3, and if so, would it be phosphorylated in response to these cytokines? Therefore, we assayed JAK3 levels in several myeloid cell lines. Both the megakaryocytic leukemia line, M07e, and the erythroleukemia cell line TF-1 (22, 23) were found to express high levels of JAK3 protein that were not significantly altered by the presence of growth factors. In contrast the myeloid lines U937 and THP-1 did not have detectable JAK3 (8, and data not shown).

The M07e cell line has been reported to proliferate in response to IL-2 or IL-4 but not IL-7 (23). Therefore, we examined whether JAK3 might be tyrosine phosphorylated upon cytokine stimulation of the endogenous IL-2 or IL-4 receptors in these cells. As shown in Fig. 1, in M07e the level of tyrosine phosphorylation of JAK3 increased dramatically in response to IL-2 or IL-4 (lanes 2 and 3) but not to GM-CSF (lane 4). Peptide competition of the JAK3 immunoprecipitation from IL-2–stimulated cells (lane 5) demonstrated the specificity of the JAK3 antibody. These filters were then stripped of antibody and probed with anti-JAK3 antibody to confirm that with the exception of lane 5, equivalent amounts of JAK3 were precipitated after cytokine treatment (Fig. 1, bottom). These data suggested that JAK3 is likely an important signaling molecule for the endogenous cytokine receptors of these myeloid cells.

JAK3 Is Expressed at Low Levels in Resting Monocytes but Is Induced during Monocyte Activation. Human peripheral blood monocytes respond to IL-2, IL-4, and IL-7 particularly after activation (16, 17, 24, 25). We previously reported that resting monocytes express little JAK3 (8). However, monocytes respond to IFN-γ or LPS with phenotypical, functional, and biochemical changes including the upregulation of several genes and heightened responses to cytokine stimulation, and
Figure 1. Tyrosine phosphorylation of JAK3 in M07e. M07e was unstimulated (lane 1) or treated with 1,000 U/ml IB2 (lanes 2 and 5), 100 U/ml IL-4 (lane 3), or 100 ng/ml GM-CSF (lane 4) for 10 min, lysed, and JAK3 was immunoprecipitated. The resulting filters were immunoblotted with monoclonal antiphosphotyrosine antibody (top), then with anti-JAK3 antibody (bottom).

JAK3 is upregulated during T cell activation (8, 16, 17, 24, 25). Therefore, we examined whether JAK3 levels might increase significantly during monocyte activation. As shown in Fig. 2A, Northern analysis demonstrated that resting monocytes express only low levels of JAK3 mRNA (lane 1) but monocytes stimulated for 18 h with IFN-γ (Fig. 2A, lane 2), LPS (lane 3), or both (lane 4) expressed high levels of JAK3 mRNA. In these experiments, JAK3 mRNA appears as two separate species due to interference of comigrating 28S RNA. Dose titration experiments showed that JAK3 mRNA could be induced with as little as 4 U/ml IFN-γ, with maximal expression at 100–500 U/ml (Fig. 2B). JAK3 mRNA increased rapidly in response to IFN-γ, detectable within 6 h, maximal by 24 h, and declining thereafter (Fig. 2C). When other cytokines were screened for the ability to upregulate JAK3 mRNA, we found that both IL-1 and TNF-α induced JAK3 mRNA, but IL-3 and GM-CSF did not (data not shown).

Because some cytokines are capable of upregulating their own receptors, we next asked whether cytokines likely to be linked to JAK3 might also be able to induce JAK3 expression. To this end, monocytes were stimulated for 48 h with IFN-γ, and/or LPS, or each of three cytokines IL-2, IL-4, and IL-7. The data shown in Fig. 3 demonstrate that, in addition to induction by IFN-γ and/or LPS, JAK3 mRNA was induced by culture with IL-2, but not IL-4 or IL-7. To verify that these cytokine treatments result in a concomitant increase in cellular JAK3 protein, lysate of similarly treated cells was immunoblotted with anti-JAK3 polyclonal antisera. Fig. 4A shows that, as suggested by the mRNA analysis, the expression of a JAK3 was induced in response to IL-2, IFN-γ, and LPS, with the latter being by far the most potent stimulus. Immunoblotting of lysates from control or LPS-stimulated cells in the presence of cognate JAK3 peptide demonstrated the specificity of the immunoblotting in monocytes (Fig. 4B). In similar experiments, JAK3 protein was found to be expressed in resting monocytes at very low levels, and detect-
Figure 3. Induction of JAK3 mRNA by IL-2 but not IL-4 or IL-7. Monocytes were stimulated for 18 h with nothing (lane 1), 1,000 U/ml IL-2, 500 U/ml IFN-γ, 100 U/ml IL-4, 100 ng/ml IL-7, or 100 ng/ml LPS (lanes 2-6, respectively). Total RNA was extracted and analyzed for JAK3 mRNA. RNA loading was quantitated using expression of G3PDH.

Figure 4. Expression of JAK3 protein after stimulation with γc cytokines. (A) Cells were stimulated as in Fig. 3, lanes 1-6, or with both 100 ng/ml LPS and 500 U/ml IFN-γ (lane 7). Postnuclear lysates were immunoblotted with JAK3 antiserum. (B) Lysates from control (lane 1) or LPS-stimulated cells (lane 2) were immunoblotted with JAK3 in the presence of cognate JAK3 peptide.

Figure 5. Tyrosine phosphorylation of JAK3 in response to IL-2, IL-4, and IL-7. Primed monocytes were either unstimulated (lane 1) or treated with 1,000 U/ml IL-2 (lane 2), 100 U/ml IL-4 (lane 3), 100 ng/ml IL-7 (lane 4), 500 U/ml IFN-γ (lane 5), or 100 ng/ml GM-CSF (lane 6) for 10 min, then lysed; immunoprecipitated JAK3 was immunoblotted with monoclonal antiphosphotyrosine antibody (top), then polyclonal anti-JAK3 (bottom).

JAK3 Phosphorylation in Response to IL-2, IL-4, and IL-7 in Activated Monocytes. Having established that monocytes express high levels of JAK3 after stimulation with IFN-γ and LPS, we asked whether JAK3 might be involved in the cytokine signal transduction pathways of human monocytes. Cells were preactivated then stimulated with various cytokines, and tyrosine phosphorylation of JAK3 was assessed. Fig. 5 shows that high levels of tyrosine phosphorylation of JAK3 were observed in response to IL-2, IL-4, or IL-7, but not GM-CSF or IFN-γ. Stripping of this blot followed by direct immunoblotting of JAK3 (Fig. 5, bottom) demonstrated that the cytokine treatments did not affect the quantity of JAK3 immunoprecipitated. In addition, as suggested by our previous findings in T cells (8, 11), preliminary data indicate that IL-4 stimulation of monocytes results in the tyrosine phosphorylation of JAK1 as well as JAK3 (data not shown). These data suggested that, in monocytes, JAK3 is coupled to three distinct cytokine receptors that use the γc chain.

Discussion

We have recently reported that the newest Janus kinase, JAK3, is coupled to the receptors for IL-2 and IL-4 in T and NK cells (7, 8, 11). We reported that JAK3 was not detectable in resting human monocytes, but others described constitutive JAK3 expression in a murine myeloid cell line (11, 20). Here we resolve this discrepancy by confirming our
JAK3 is tyrosine phosphorylated in response to IL-2 (11). Moreover, we demonstrate that JAK3 in monocytes is highly regulated in monocytes. Whereas resting monocytes express very low levels of JAK3, we show that potent monocyte activators such as IL-2, IFN-γ, and LPS all induce JAK3 expression, and that some human factor-dependent myeloid cell lines constitutively express JAK3. In addition, we demonstrate that JAK3 in monocytes is tyrosine phosphorylated in response to IL-2, IL-4, or IL-7 treatment.

The Janus family is now known to be involved in the signal transduction pathways of several cytokines that use receptors of the hematopoietin family (3, 4). Currently, the family consists of four proteins JAK1, JAK2, and Tyk2, all ubiquitously expressed, and the most recently described member, JAK3, a kinase predominantly expressed in NK and activated T cells (8, 7, 11, 20, 26). The induction of JAK3 in monocytes by IFN-γ and LPS that we report here is not surprising given the potent effects these agents have on monocyte function. However, the induction of JAK3 by IL-2 becomes the first demonstration of JAK3 protein regulation by a cytokine that signals via this kinase. These data suggest that IL-2 can induce JAK3 even in resting monocytes in which JAK3 expression is minimal. Whereas at present we can only speculate as to how signaling occurs under this circumstance, a possible explanation for this apparent paradox is that the responses of a population of resting monocytes to IL-2 or IL-4 represents an autocrine response begun by a subset of preactivated monocytes expressing higher levels of JAK3. Alternatively, these data could indicate the participation of kinases other than JAK3 in the cytokine response of resting monocytes. Both of these possibilities are currently under investigation. Regardless, a model requiring stimulation of monocytes to achieve full IL-2 responsiveness is supported by the fact that relatively high amounts of IL-2 are needed to stimulate resting monocytes as compared to preactivated monocytes (27). Moreover, preactivation of monocytes with IL-2, IFN-γ, or LPS induces both JAK3 and IL-2Rα chains (28, 29) resulting in higher levels of high affinity IL-2 receptor, more JAK3 for signal transduction, and as a result, an increase in the sensitivity of monocytes to stimulation with low doses of IL-2.

In addition to activated lymphoid cells, high JAK3 expression has been reported in the murine myeloid cell line 32D (11). When these cells are transfected with the IL-2 receptor, JAK3 is tyrosine phosphorylated in response to IL-2 (11). Here we have demonstrated that the M07e cell line, derived from a patient with megakaryoblastic leukemia (30), expresses JAK3 constitutively. Moreover, our data show that these cells use JAK3 in the signal transduction pathways of their endogenous IL-2 and IL-4 receptors but not those for GM-CSF or IFN-γ. Taken together, these data suggest an important role for JAK3 in the cytokine responses of this myeloid leukemia cell line. It should be noted, however, that a screen of 40 primary marrow aspirates from patients with acute myeloid leukemia detected only four that expressed significant levels of JAK3 protein (data not shown). The overall importance of JAK3 in myeloid leukemia is under further investigation.

Our current findings that, in addition to IL-2 and IL-4, JAK3 is tyrosine phosphorylated in response to IL-7, demonstrate that JAK3 may be a critical signal transduction molecule in the pathway of all of the cytokine receptors currently known to use the γc chain. The established associations of JAK3 with IL-2, IL-4, and IL-7 signaling dictate that further elucidation of the specific roles of JAK3 in monocyte cytokine responses, such as the identification of specific substrates and intracellular interactions, should lead to a better understanding of the overall regulation of monocyte function during an immune response.

The cytokines IL-2, IL-4, and IL-7 play important but divergent roles in monocyte development and maturation, making it important to explore the differences among the signaling pathways elicited by each of these factors. Clearly, the use of JAK3 by all known γc receptors suggests that, at the level of JAK kinases, the signal transduction pathways for these cytokines are similar. However, cytokine-induced tyrosine phosphorylation of JAK kinases is followed by tyrosine phosphorylation of latent SH2, SH3 domain containing transcription factors known as signal transducers and activators of transcription, or STAT proteins (31, 32). Therefore, the tyrosine phosphorylated STAT or STAT-like proteins that result from the binding of various γc receptors may, in part, determine signal specificity. Consistent with this hypothesis, apparently distinct tyrosine phosphorylated DNA binding proteins now are known to be associated with both IL-2 and IL-4 stimulation (33-35). Thus, whereas JAK3 and γc represent similar mechanisms by which IL-2, IL-4, and IL-7 signal, the points at which these pathways diverge towards their cytokine-specific effects will require further investigation.

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References

1. Miyajima, A., T. Kitamura, N. Harada, T. Yokota, and K. Ariai. 1992. Cytokine receptors and signal transduction. Annu. Rev. Immunol. 10:295–331.

2. Cosman, D. 1993. The hematopoietin receptor superfamily. Proc. Natl. Acad. Sci. USA. 9:95–106.

3. Ziemiecki, A., A. Harpur, and A.F. Wilks. 1994. JAK protein tyrosine kinases: their role in cytokine signalling. Trends Cell Biol. 4:207–212.

4. Ihle, J.N., B.A. Witthuhn, F.W. Quelle, K. Yamamoto, W.E. Ihle, F.W. Quelle, K. Yamamoto, W.E. Ihle, B.A. Witthuhn, and F.W. Quelle. 1994. Janus kinase in signalling in interleukins 2 and 4 in lymphoid and myeloid cells. Nature (Lond.). 370:153–157.

5. Sadowski, M.Z. Gilman, and J.E. Darnell. 1993. Polypeptide recruitment of the interleukin-2 receptor in human monocytes and modulated by interleukin-2, interferon-γ and transforming growth factor beta 1. Blood. 83:3462–3467.

6. Paul, W.E. 1993. Interleukin-4: a prototypic immunoregulatory lymphokine. Blood. 77:1859–1870.

7. Vitale, A.A., J.P.G. Klomp, B.A. Yard, J.E. de Vries, and C.G. Figdor. 1988. Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. J. Immunol. 140:1548–1554.

8. Rane, S.G., and E.P. Reddy. 1994. JAK3: a novel JAK kinase associated with terminal differentiation of hematopoietic cells. Oncogene. 9:2415–2423.

9. Nathan, C.F. 1987. Secretory products of macrophages. J. Exp. Med. 166:251–262.

10. Urdal, D.L., V. Price, H.M. Sassenfeld, D. Cosman, S. Gillis, and L.S. Park. 1989. Molecular characterization of colony-stimulating factors and their receptors: human interleukin-3. Ann. N.Y. Acad. Sci. 554:1564–1572.

11. Alderson, M.R., T.W. Tough, S.F. Ziegler, and K.H. Grabstein. 1991. Interleukin 7 induces cytokine secretion and tumoricidal activity by human peripheral blood monocytes. J. Exp. Med. 173:923–930.

12. Bosco, M.C., I. Espinoza-Delgado, M. Schwabe, S.M. Russell, W.J. Leonard, D.L. Longo, and L. Varesio. 1994. The gamma subunit of the interleukin-2 receptor is expressed in human monocytes and modulated by interleukin-2, interferon-γ and transforming growth factor beta 1. Blood. 83:3462–3467.
signalling to the nucleus through tyrosine phosphorylation of Jak and Stat proteins. Nature (Lond.). 366:580-583.
33. Schindler, C., H. Kashleva, A. Pernis, R. Pine, and P. Rothman. 1994. STF-IL-4: a novel IL-4-induced signal transducing factor. EMBO (Eur. Mol. Biol. Organ.) J. 13:1350–1356.
34. Gilmore, K.C., and N.C. Reich. 1994. Receptor to nucleus signaling by prolactin and interleukin, via activation of latent DNA-binding factors. Proc. Natl. Acad. Sci. USA. 91:6850–6854.
35. Hou, J., U. Schindler, W.J. Henzel, T.C. Ho, M. Brasseur, and S.L. McKnight. 1994. An interleukin-4-induced transcription factor: IL-4 stat. Science (Wash. DC). 265:1701–1706.