

Activation of early response genes and cell proliferation by human interleukin-3, granulocyte-macrophage colony-stimulating factor, and interleukin-5 receptors: Comparison with human interleukin-4 receptor signaling

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Interleukin (IL)-3, granulocyte-macrophage colony-stimulating factor, and IL-5 receptors (IL-3R, GMR, and IL-5R) are composed of the α chain specific to each and the common β chain, and both the α and β subunits are members of the cytokine receptor superfamily. We previously showed that the high-affinity human GMR reconstituted by cotransfecting the α and β chain cDNA clones transduces signals in response to hGM-CSF to activate transcription of c-fos, c-jun, and c-myc proto-oncogenes in mouse proB cell line BA/F3 or in mouse fibroblast NIH3T3 cells. These results indicated that molecules, such as tyrosine kinase, unique to hematopoietic cells are not essential to transduce signals. In this study, the function of the α subunit of GMR was compared with those of IL-3R and IL-5R by cotransfecting human cDNAs encoding the α subunit of IL-3R or IL-5R and the common β subunit into BA/F3 or NIH3T3 cells. We found that the reconstituted human IL-3R, in response to hIL-3, transduced signals to activate transcription of c-fos promoter and induced DNA synthesis in both types of cells in a manner similar to hGMR. Likewise, hIL-5 activates c-fos promoter in transfected NIH3T3 cells expressing hIL-5R. These results indicated that the α subunits of IL-3R and IL-5R have properties similar to those of the GMR α subunit. In contrast, transfected human IL-4 receptor (hIL-4R) cDNA, which weakly activated c-fos promoter and induced DNA synthesis in BA/F3 cells, failed to elicit these activities in NIH3T3 cells in response to hIL-4. It is likely that NIH3T3 cells lack some component or components required for signal transduction by hIL-4R. (J ALLERGY CLIN IMMUNOL 1994;94:605-11.)

Key words: Transfected human IL-4 receptor, receptor signaling, signal transduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-3 act on a wide variety of hematopoietic cells to stimulate proliferation and differentiation.^{1,2} In contrast, the action of IL-5 is more restricted and acts mainly on eosinophils, some B cells, and basophils.^{3,4} IL-3 and GM-CSF act in a species-specific manner between mice and human beings,

Abbreviations used

β c:	Common β subunit
FCS:	fetal calf serum
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
GMR:	GM-CSF receptor
IL:	Interleukin

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whereas activities of mouse (m)IL-5 and human (h)IL-5 are interchangeable. Molecular cloning of cDNAs encoding IL-3 receptor (IL-3R) and GM-CSF receptor (GMR) has revealed that these two receptor systems have the α chain specific to each and the common β chain both in the human and the mouse systems.⁵ It was also shown that the same β chain associates with the IL-5R α chain to

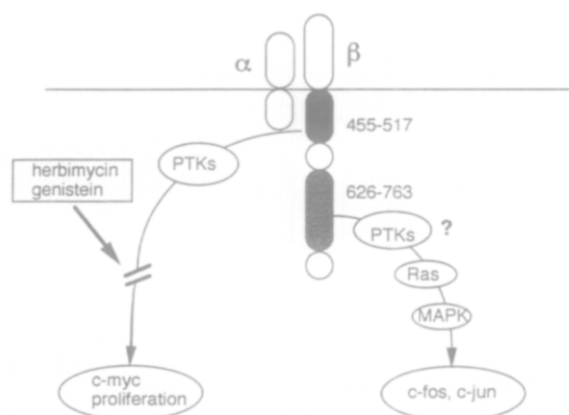


FIG. 1. Scheme for two distinct signal transduction pathways controlled by the hGMR β subunit in BA/F3 proB cells.

form the high-affinity IL-5R. The α subunit of GMR, IL-3R, or IL-5R binds the specific ligand with low affinity. The β subunit does not bind any cytokine by itself but forms high-affinity receptors when complexed with the α subunits. In human beings only one β subunit common to GMR, IL-3R, and IL-5R has been recognized, whereas two distinct β subunits are found in mice (i.e., AIC2A, the β subunit for IL-3R, and AIC2B, the common β subunit [β c] shared by GMR, IL-3R, and IL-5R).^{6,7}

GM-CSF or IL-3 induces mRNAs of several early response genes (e.g., *c-fos*, *c-jun*, *c-myc*), tyrosine phosphorylation of cellular substrates, and cell proliferation. Neither the α nor the β subunit of IL-3R and GMR has structural features characteristic of a tyrosine kinase or a serine/threonine kinase domain, even though IL-3 or GM-CSF induce rapid tyrosine phosphorylation of several proteins including p140, p90, and p60 proteins. These results indicated that the tyrosine kinase activated by IL-3 or GM-CSF is distinct from IL-3R or GMR. In previous studies we have shown that both the α and β subunits are essential and sufficient to reconstitute high-affinity human (h)GMR on various mouse cell lines such as proB cell (BA/F3), T cell (CTLL2), and fibroblast (NIH3T3).^{6,8} A BA/F3 cell proliferates strongly in response to mIL-3 and weakly in response to mIL-4; however, CTLL-2 and NIH3T3 do not respond to either mIL-3 or mIL-4. Using tyrosine kinase inhibitors and cytoplasmic deletion mutants of the β c, we have shown that there are at least two distinct pathways downstream of GMR in BA/F3 cells.^{9,10} One is essential for proliferation, leads to *c-myc* and *pim-1* gene activation, and is sensitive to herbimycin

and genistein. Activation of this pathway depends on the cytoplasmic region between amino acid positions 455 and 517 of the β c. The other, which leads to the activation of *c-fos* and *c-jun* genes, depends on the region between amino acid positions 626 and 763 of the β c. Activation of these genes is only partially sensitive to herbimycin, and is resistant to genistein. This region is also required for activation of ras, raf-1, MAP kinase, and p70 S6 kinase (Fig. 1).

However, several important questions regarding the IL-3R/GMR/IL-5R system, such as the role of the α subunit and the nature of the signals unique to each, remain unanswered. Inasmuch as the common signals are likely to be mediated by the β subunit shared by all, the signals unique to each receptor may be mediated by the α subunit. However, the structure of the active form of the receptors [i.e., (α , β), (α , β)₂, or other combinations] and the exact nature of the contribution of the α subunit in signalling are not clearly understood. In this study, we compared the properties of the α subunits of the hIL-3R, hGMR, or hIL-5R by reconstituting a high-affinity receptor in a mouse proB cell line BA/F3 and the NIH3T3 fibroblast after transfection of the cDNAs encoding the α and the β subunits.

IL-4 is a cytokine that displays pleiotropic biologic effects. It functions as a costimulator of proliferation and differentiation of B cells, T cells, thymocytes, and mast cells; and it induces class II major histocompatibility antigens on resting B cells, stimulates IgG₁ and IgE secretion by activated B cells, and regulates the expression of Fcε receptors (CD23) and other cell surface molecules on B and T cells.¹ The signal transduction pathway for IL-4 receptor (IL-4R) remains unclear. In human B cells IL-4 has been shown to induce an immediate and transient rise in inositol 1, 4, 5-triphosphate and Ca²⁺ levels, followed by a sustained elevation of cAMP. Inositol 1, 4, 5-triphosphate mobilizes an intracellular store of calcium and diacylglycerol, which is an activator of protein kinase C. In mouse B cells costimulation with IL-4 and nonmitogenic concentration of anti-IgM antibody induces protein kinase C translocation from cytosol to membrane and drives resting B cells to DNA synthesis, whereas stimulation by either of them alone does not. IL-2, IL-3, or GM-CSF activates ras protein, whereas IL-4 has no effect on ras-bound guanosine diphosphate/guanosine triphosphate.¹¹ Protein tyrosine phosphorylation is induced by IL-4 in the mouse T-cell line HT2,¹² myeloid cell lines FDC-P1 and FDCP2,¹³ and B cells.¹⁴ However, no changes in

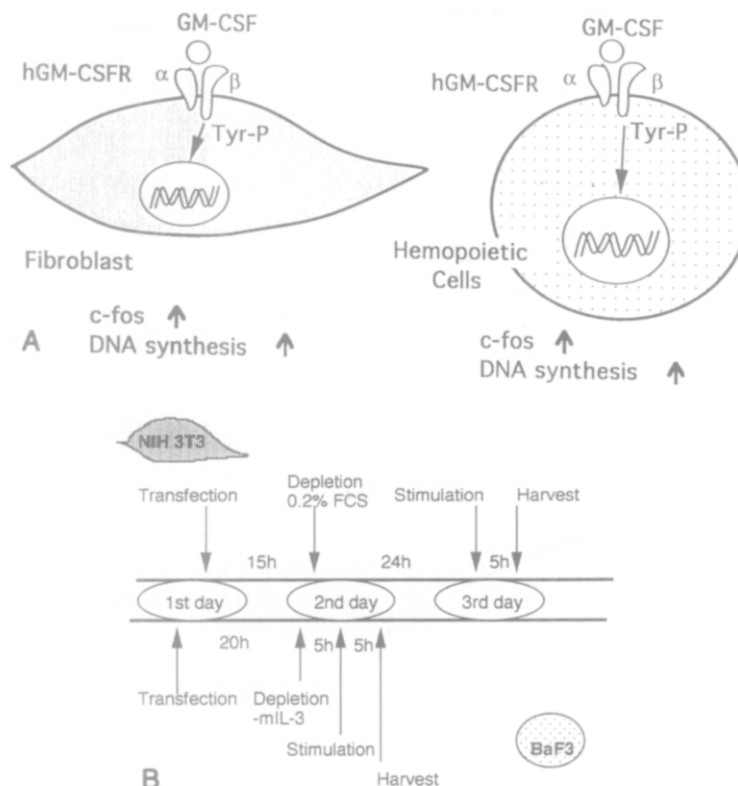


FIG. 2. **A**, Signal transduction by high-affinity hGMR reconstituted in BA/F3 cells and NIH3T3 cells. **B**, Protocol for transient transfection assay of *c-fos* luciferase activity.

phosphorylation have so far been detected in human cell lines, and IL-4, rather, caused rapid dephosphorylation from tyrosine residues of an 80 kd phosphoprotein.¹⁵ Deletion analysis of the cytoplasmic domain of the hIL-4R in BA/F3 cells revealed that the region located between amino acid residues from 433 to 473 (counted from the C-terminus) is critical for cell growth.¹⁶ Only few reports describe the induction of early response genes by IL-4 in mouse B cells.¹⁷ These results indicate that the nature of the intracellular signals induced by IL-4R is substantially different from the nature of those induced by GMR. In this study, we also compared the properties of hIL-4R with those of hGMR, hIL-3R, and hIL-5R in hematopoietic cells and fibroblasts.

EXPERIMENTS

Activation of early response genes and proliferation by hGM-CSF in NIH3T3 cells transfected with hGMR

GM-CSF plays a critical role in growth and differentiation of myeloid cells. High-affinity hGMR reconstituted in a proB cell line, BA/F3, by cotransfecting the α and β chain cDNAs was

capable of transducing growth-promoting signals. It has been assumed that GMR requires a component or components unique to hematopoietic cells for signal transduction. However, by using NIH3T3 fibroblasts, which normally do not express GMR, we found that the reconstituted hGMR transduces signals in NIH3T3 cells in response to hGM-CSF to activate transcription of *c-fos*, *c-jun*, and *c-myc* proto-oncogenes. hGM-CSF also induces protein tyrosine phosphorylation and DNA synthesis in both cell types. The ability of hGMR to transduce signals was affected by inhibitors of tyrosine kinase.⁹ These results indicated that the hGMR is functional in fibroblasts, that signal transduction via the hGMR in fibroblasts involves tyrosine kinase(s), and that the association of hGMR with factor(s) specific to hematopoietic cell lineage is not essential to transduce growth-promoting signals (Fig. 2, A).⁸

hIL-3 or hIL-5 activates *c-fos* promoter in NIH3T3 cells expressing hIL-3R or hIL-5R

The ability of the α subunit of hIL-3R or hIL-5R to transduce growth-promoting signals was compared in the same manner as of hGMR in

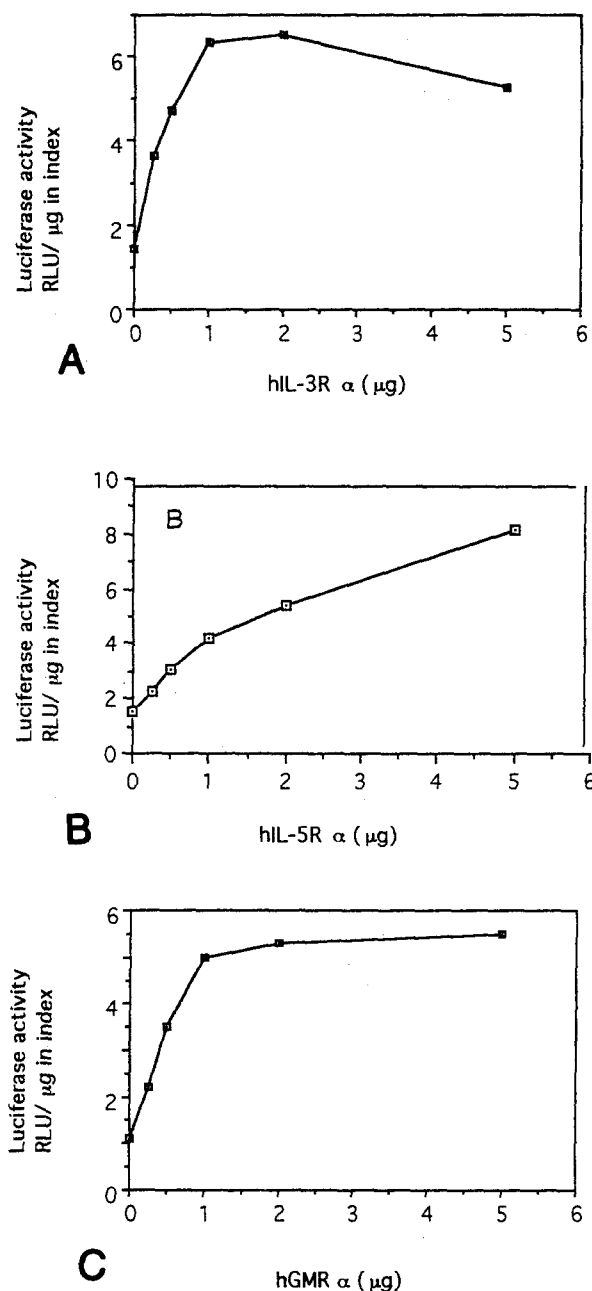


FIG. 3. Activation of *c-fos* promoter by hIL-3, hIL-5, or hGMR in NIH3T3 fibroblasts transfected with the common β subunit and the α subunit of hIL-3R (A), hIL-5R (B), and hGMR (C). The cDNA encoding the α subunit of hIL-3R, hIL-5R, or hGMR; the cDNA encoding the common β subunit (β c); and *c-fos* promoter-luciferase reporter plasmid were introduced into NIH3T3 cells by a transient transfection method. The cells were harvested, and luciferase activity was determined as described previously.⁸

transient transfection assay. For this purpose, we cotransfected cDNA encoding the α subunit of hIL-3R, hIL-5R, or hGMR with the β subunit cDNA into NIH3T3 cells. After transfection, cells

were cultured overnight in medium containing 10% fetal calf serum (FCS), depleted of FCS (0.2%) for 24 hours, and then stimulated with appropriate cytokine as indicated. After 5 hours, cells were harvested and assayed for luciferase activity (Fig. 2, B). We found that the high-affinity hIL-3R and hIL-5R, similar to hGMR, are capable of activating *c-fos* promoter linked to luciferase reporter gene in NIH3T3 cells (Fig. 3) when saturated amounts of the β chain cDNA (5 mg for 4×10^6 cells) and hIL-3 (10 ng/ml) or hIL-5 (5 ng/ml) were used. The level of luciferase activity increased in a manner dependent on the amount of the α subunit DNA transfected. Under the standard assay conditions in NIH3T3 cells (4×10^6 cells), the amounts of the α subunit cDNA required to achieve maximum expression of the luciferase reporter gene were 2 mg, 5 mg, and 1 mg for hIL-3R (Fig. 3, A), IL-5R (Fig. 3, B), and GMR (Fig. 3, C), respectively.

hIL-3 induces proliferation of NIH3T3 cells expressing high-affinity hIL-3R

BA/F3 cells extensively proliferate in response to mIL-3, and likewise, hIL-3 stimulates DNA synthesis of BA/F3 cells expressing high-affinity hIL-3R (unpublished data). We next attempted to determine whether hIL-3 induces proliferation of NIH3T3 cells expressing high-affinity hIL-3R. We used stable transfectants of NIH3T3 fibroblasts expressing both high-affinity hIL-3R and hGMR. Cells were depleted of FCS for 24 hours, then exposed to hIL-3, hGM-CSF, or FCS; and the amount of tritiated-thymidine incorporated into DNA was determined. We found that hIL-3 induced cell proliferation in a manner similar to hGM-CSF. The extent of proliferation was slightly higher than that induced by hGM-CSF but lower than that induced by FCS (Fig. 4).

The α subunit of hIL-3R or hIL-5R has a function similar to that of hGMR

Taken together, these results indicate that the high-affinity hIL-3R and hIL-5R reconstituted in NIH3T3 cells are functional and transduce signals similar to the reconstituted high-affinity hGMR. No significant difference was found among the α subunits of GMR, IL-3R, and IL-5R in their ability to activate *c-fos* promoter. Therefore it appears that NIH3T3 fibroblasts, like myeloid cells, contain signal-transducing molecules that can effectively interact with GMR, IL-3R, and IL-5R. Others reported that hGMR was capable of initiating signal transduction but did not induce significant proliferation in transfected NIH3T3

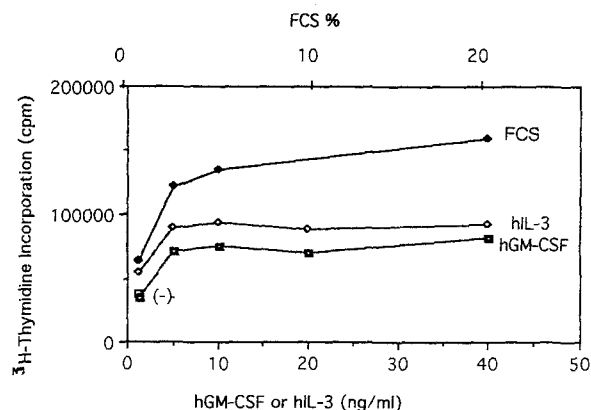


FIG. 4. Induction of cell proliferation by hIL-3 or hGM-CSF of NIH3T3 cells stably transfected with the common β subunit and the α subunits of hIL-3R and hGMR. The plasmids that had neomycin and hygromycin genes and cDNAs encoding the α subunits of hGMR, hIL-3R, and the common β subunit were introduced by the calcium phosphate method into NIH3T3 cells. The cells stably transfected with both hIL-3R and hGMR were selected with 0.6 mg/ml G418 and 0.6 mg/ml hygromycin.⁶

cells.¹⁸ The reason for this discrepancy is unclear, but it is possible that the level of either the hGMR α subunit or the hGMR β subunit expressed on stable transfectant was below the threshold required for cell growth, even though hGM-CSF induced *c-fos* and *c-myc* mRNAs and protein tyrosine phosphorylation.

Role of the α subunits of GMR, IL-3R, and IL-5R

Deletion analyses have indicated that the cytoplasmic domains of both α and β subunits were required for GMR signal transduction.^{9,10} We further analyzed the role of the cytoplasmic domains of the α and β subunits by constructing chimeric subunits of hGMR. Both subunits, hGMR(α/β) and hGMR(β/α), were generated by replacing the cytoplasmic domains of the α and β subunits of hGMR with those of the β and α subunits, respectively. Reconstitution studies have indicated that the high-affinity hGMR(α/β) composed of the α/β and β subunits, which carries no cytoplasmic portion of the α subunit, was functional.¹⁹ Thus the cytoplasmic domain of the β subunit is sufficient to transduce growth-promoting signals, and the cytoplasmic domain of the α subunit is capable of forming a functional hGMR. On the basis of these observations, we proposed the following events for the activation of hGMR. First, hGM-CSF induces heterodimerization of the α and β subunits. Second, oligomerization of this ternary complex results in forma-

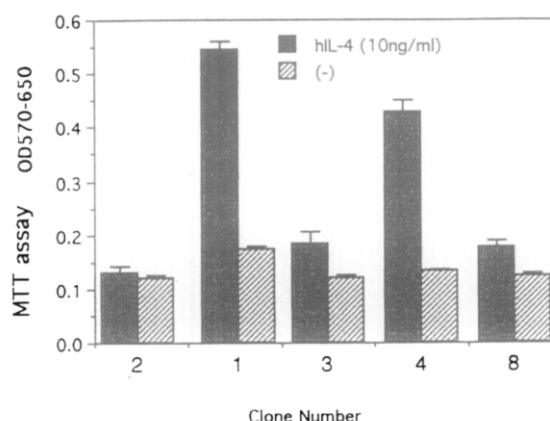


FIG. 5. Stimulation of cell proliferation by hIL-4 of BA/F3 proB cells expressing hIL-4R. hIL-4R cDNA and the neomycin gene were introduced into BA/F3 cells, and cells stably transfected with hIL-4R cDNA were selected with 0.8 to 1.2 mg/ml G418. Several independent clones were examined for their ability to grow in response to hIL-4.

tion of the oligomeric β subunit. It is likely that the cytoplasmic domain of the α subunit is required to support oligomerization of the β subunit. Third, growth-promoting signals are transduced through the cytoplasmic domain of the β subunit in oligomeric form. If this is found to be the case, the results described in this article suggest that the cytoplasmic domains of the α subunits of GMR, IL-3R, and IL-5R have abilities in common to support oligomerization of the β subunit. This does not exclude the possibility that the cytoplasmic domains of the α subunits of these receptors have functions unique to each.

hIL-4 activates *c-fos* promoter and induces DNA synthesis in BA/F3 cells but not in NIH3T3 cells expressing hIL-4R

Next, we examined signal transduction by hIL-4 in BA/F3 cells and NIH3T3 cells transfected with hIL-4R cDNA. In a transient transfection assay, hIL-4 weakly activated the *c-fos* promoter transfected with hIL-4R cDNA in BA/F3 cells (data not shown). BA/F3 cells proliferated weakly in response to mIL-4. The ability of hIL-4R to promote cell proliferation in response to hIL-4 was determined with several stable transfectants of BA/F3 cells expressing hIL-4R. All of the transfectants expressing hIL-4R proliferated in response to hIL-4 (Fig. 5: clones 1, 3, 4, 8). The extent of proliferation varied considerably between clones. Some BA/F3 cells transfected with hIL-4R cDNA (Fig. 5, clone 2) but did not express hIL-4R and failed to proliferate as expected. Interestingly, in contrast to the hGMR

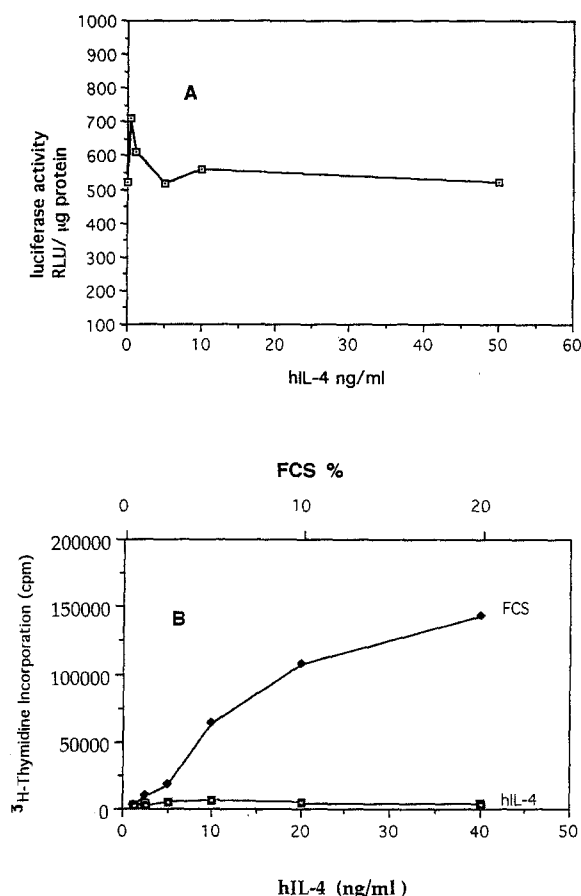


FIG. 6. hIL-4 neither activates the *c-fos* promoter (**A**) nor stimulates cell proliferation (**B**) in NIH3T3 cells transfected with hIL-4R. The hIL-4R cDNA was introduced into NIH3T3 cells by the electroporation method, and stable transfectants were selected with hygromycin at 100 mg/ml.

system, hIL-4 neither activated *c-fos* promoter (Fig. 6, A) nor induced DNA synthesis (Fig. 6, B) in NIH3T3 fibroblasts stably transfected with hIL-4R cDNA. hIL-4 also did not stimulate *c-fos* promoter in NIH3T3 cells expressing hIL-4R in a transient transfection assay (data not shown). These results suggest that NIH3T3 cells lack a certain component, which is present in BA/F3 cells and is required for signal transduction by hIL-4R. Alternatively, some inhibitor for signal transduction of hIL-4R exists in NIH3T3 cells, which is absent in BA/F3 cells. It should be noted that we, as well as others,^{20, 21} have recently shown that IL-4R and IL-2R share the IL-2R γ subunit to support proliferation of T cells. Therefore it is tempting to speculate that one of the missing components in NIH3T3 cells for hIL-4R signaling is the hIL-2R γ subunit. In BA/F3 pro-B cells, hIL-4R may interact with the mIL-2R γ subunit

to form an hIL-4R complex with higher affinity for the ligand. However, hIL-4 was unable to activate *c-fos* promoter in NIH3T3 cells expressing both the hIL-4 and the hIL-2R γ subunit (Chen et al. Unpublished results). It has also been suggested that IL-4R and IL-13R have some component or components in common,²² although the exact relationship between the IL-4R and the IL-13R systems is yet to be clarified. These results, along with the studies on IL-4-associated proteins, suggest that the molecular composition of the IL-4R system is complicated.^{23, 24} Clearly, more work is necessary to elucidate the structure and function of the active form of IL-4R in various types of cells.

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REFERENCES

1. Arai KI, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T. Cytokines: coordinators of immune and inflammatory responses. *Ann Rev Biochem* 1990;59:783-836.
2. Miyajima A, Kitamura T, Harada N, Yokota T, Arai K. Cytokine receptors and signal transduction. *Ann Rev Immunol* 1992;10:295-331.
3. Lopez AF, Eglinton JM, Lyons AB, et al. Human interleukin-3 inhibits the binding of granulocyte-macrophage colony-stimulating factor and interleukin-5 to basophils and strongly enhances their functional activity. *J Cell Physiol* 1990;145:69-77.
4. Lopez AF, Vadas MA, Woodcock J, et al. Interleukin-5, interleukin-3, and granulocyte-macrophage colony-stimulating factor cross-compete for binding to cell surface receptors on human eosinophils. *J Biol Chem* 1991;266:24741-7.
5. Hayashida K, Kitamura T, Gorman DM, Arai KI, Yokota T, Miyajima A. Molecular cloning of a second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF): reconstitution of a high-affinity GM-CSF receptor. *Proc Natl Acad Sci U S A* 1990;87:9655-9.
6. Kitamura T, Hayashida K, Sakamaki K, Yokota T, Arai K, Miyajima A. Reconstitution of functional receptors for human granulocyte/macrophage colony-stimulating factor (GM-CSF): evidence that AIC2B is a subunit of murine GM-CSF receptor. *Proc Natl Acad Sci U S A* 1991;88:5082-6.
7. Kitamura T, Sato N, Arai K, Miyajima A. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for human IL-3 and GM-CSF receptors. *Cell* 1991;66:1165-74.
8. Watanabe S, Mui ALF, Muto A, et al. Reconstituted human granulocyte-macrophage colony-stimulating factor receptor transduces growth-promoting signals in mouse NIH 3T3 cells: comparison with signalling in BA/F3 pro-B cells. *Mol Cell Biol* 1993;13:1440-8.

9. Watanabe S, Muto A, Yokoda T, Miyajima A, Arai KI. Differential regulation of early response genes and cell proliferation through the human granulocyte macrophage colony-stimulating factor receptor: selective activation of the *c-fos* promoter by genistein. *Mol Biol Cell* 1993;4:983-92.
10. Sato N, Sakamaki K, Terada N, Arai K, Miyajima A. Signal transduction by the high affinity GM-CSF receptor: two distinct cytoplasmic domains of the common β subunit responsible for different signaling. *EMBO J* 1993;12:4181-9.
11. Satoh T, Nakafuku M, Miyajima A, Kaziro Y. Involvement of *ras* in signal transduction pathways from interleukin-2, interleukin-3, and granulocyte-macrophage colony stimulating factor, but not from interleukin-4. *Proc Natl Acad Sci U S A* 1991;88:3314-8.
12. Izuhara K, Harada N. Interleukin-4 (IL-4) induces protein tyrosine phosphorylation of the IL-4 receptor and association of phosphatidylinositol 3-kinase to the IL-4 receptor in a mouse T cell line, HT2. *J Biol Chem* 1993;268:13097-102.
13. Wang LM, Keegan AD, Li W, et al. Common elements in interleukin-4 and insulin signaling pathways in factor-dependent hematopoietic cells. *Proc Natl Acad Sci U S A* 1993;90:4032-6.
14. McGarvie GM, William C. Appearance of a novel plasma membrane phosphoprotein following culture of resting murine B lymphocytes with recombinant interleukin-4. *Immunol Lett* 1989;22:221-6.
15. Mire-Shuis AR, Thorpe R. Interleukin-4 proliferative signal transduction involves the activation of a tyrosine-specific phosphatase and the dephosphorylation of an 80-kDa protein. *J Biol Chem* 1991;266:18113-8.
16. Harada N, Yang G, Miyajima A, Howard M. Identification of an essential region for growth signal transduction in the cytoplasmic domain of the human interleukin-4 receptor. *J Biol Chem* 1992;267:22752-8.
17. Klemsz MJ, Justement LB, Palmer ED, Cambier JC. Induction of *c-fos* and *c-myc* expression during B cell activation by IL-4 and immunoglobulin ligands. *J Immunol* 1989;143:1032-9.
18. Eder M, Griffin JD, Ernst TJ. The human granulocyte-macrophage colony-stimulating factor is capable of initiating signal transduction in NIH3T3 cells. *EMBO J* 1993;12:1647-56.
19. Muto A, Watanabe S, Miyajima A, Yokota T, Arai K. High affinity chimeric human GM-CSF receptor carrying the cytoplasmic domain of the β subunit but not the α subunit transduces growth promoting signals in Ba/F3 cells. (submitted)
20. Kondo M, Takeshita T, Ishii N, et al. Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. *Science* 1993;262:1874-7.
21. Russell SM, Keegan AD, Harada N, et al. Interleukin-2 receptor γ chain: a functional component of the interleukin-4 receptor. *Science* 1993;262:1880-3.
22. Zurawski SM, Vega F Jr, Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *EMBO J* 1993;12:2663-70.
23. Foxwell BM, Woerly G, Ryffel B. Identification of interleukin-4 receptor-associated protein and expression of both high- and low-affinity binding on human lymphoid cells. *Eur J Immunol* 1989;19:1637-41.
24. Galizzi JP, Castle B, Djossou O, et al. Purification of a 130-kDa T cell glycoprotein that binds human interleukin-4 with high affinity. *J Biol Chem* 1990;265:439-44.