REVIEW ARTICLE

Receptors for Granulocyte-Macrophage Colony-Stimulating Factor, Interleukin-3, and Interleukin-5

By Atsushi Miyajima, Alice L.-F. Mui, Toshiya Ogorochi, and Kazuhiro Sakamaki

Cytokines are actively involved in hematopoiesis by regulating proliferation, differentiation, and cellular functions of various lineages of hematopoietic cells. Two fundamental characteristics for cytokines have been noticed, i.e., each cytokine exhibits pleiotropic functions on different target cells and a subset of cytokines often shows similar functions on the same target cell. The structures of the high-affinity receptors for these cytokines shown by molecular cloning provide a basis for the functional redundancy. Unlike growth factor receptors with an intrinsic tyrosine kinase, the high-affinity receptors for hematopoietic cytokines consist of multiple subunits and the receptors for a subset of cytokines with similar functions share the common component essential for their signal transduction. This review will discuss recent findings concerning the structure, expression, and function of the receptors for a subset of hematopoietic cytokines, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5, which exhibit similar biologic functions in their common target cells.

Common Features of GM-CSF, IL-3, and IL-5

IL-3 stimulates the development of various lineages of hematopoietic cells by interacting with immature multipotential hematopoietic progenitors as well as lineage-committed progenitors including granulocytes, macrophage, eosinophils, mast cells, megakaryocytes, and erythroid cells (Table 1). Therefore, IL-3 is capable of stimulating colony formation of multiple lineages and is thus also known as multi-colony stimulating factor (multi-CSF). GM-CSF was originally defined as a factor that stimulates colony formation of granulocyte and macrophages. However, subsequent studies with recombinant GM-CSF have shown that GM-CSF interacts with much broader range of lineages as well as earlier stages of hematopoietic cells (Table 1). Thus, IL-3 and GM-CSF display many similar biologic activities in a wide variety of cells. While IL-5 was originally cloned as a B-cell differentiation factor in mice, it has a major role in development of eosinophils in both mice and humans. IL-3, GM-CSF, and IL-5 exhibit similar actions on eosinophils and basophils.

Although there is no significant amino acid sequence homology among these three cytokines, they exhibit a number of similarities in addition to their biologic functions. First, IL-3, GM-CSF, and IL-5 are cytokines with four α-helices and their gross tertiary structures are similar. Second, the genomic organization of these genes is similar and they are closely linked on human chromosome 5 and mouse chromosome 11. Third, they are the major hematopoietic growth factors produced by activated T cells and mast cells. Fourth, they induce protein phosphorylation of similar molecules. Finally, binding of IL-3 to its receptor is competed by GM-CSF and vice versa. Likewise, the IL-5 binding to the receptor is also competed with either IL-3 or GM-CSF. The binding cross-competition among these three cytokines led to the speculation that they might share the same receptor. Curiously, the binding cross-competition among these cytokines was reported only in the human hematopoietic cells, but not in the mouse cells. This discrepancy between mice and humans is now explained by the unique receptor subunit that is found only in the mouse cells as described below.

The Mouse IL-3 receptor

Molecular cloning of the receptor subunits was initiated by isolating a cDNA encoding a mouse IL-3 (mIL-3) binding protein, AIC2A, which is now referred to as the β subunit of the IL-3 receptor, βIL-3. The AIC2A cDNA was cloned by expression using the anti-Aic2 antibody that was raised against IL-3-dependent cell line IC2 and that partially inhibited IL-3 binding. The AIC2A cDNA encodes a single transmembrane protein of 878 amino acids including a signal sequence. The mature AIC2A protein is a glycoprotein of about 120 Kd. The intracellular domain of 413 amino acid residues has no known consensus sequence for signaling molecules such as kinases. AIC2A is a member of the type I cytokine receptor superfamily that includes the β and γ subunits of the IL-2 receptor and the receptors for IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, GM-CSF, G-CSF, erythropoietin (EPO), leukemia inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF). They have a common structural motif in their extracellular domains. This motif consists of about 200 amino acid residues with two fibronectin type III modules. Each module consists of seven antiparallel β-strands. There are four well-conserved cysteine residues in the N-terminal module and a unique sequence Trp-Ser-X-Trp-Ser (WSXWS) in the C-terminal module (Fig 1). The AIC2A protein has two repeats of the motif in the extracellular domain (Fig 1). The AIC2A protein binds mIL-3 with only low affinity (kd = 10 to 20 nmol/L) and mIL-3 dissociates rapidly from AIC2A (T1/2 = 4 minutes). These binding characteristics...
Table 1. Major Biologic Activities of IL-3, GM-CSF, and IL-5

<table>
<thead>
<tr>
<th>Biologic Activities</th>
<th>IL-3</th>
<th>GM-CSF</th>
<th>IL-5</th>
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<tbody>
<tr>
<td>Stimulation of mast cell growth</td>
<td>+ (M)</td>
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<td>-</td>
</tr>
<tr>
<td>Stimulation of CFU-S</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blast cell colony formation</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Mixed cell colony formation</td>
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<tr>
<td>GM-colony formation</td>
<td>+</td>
<td></td>
<td>+</td>
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<tr>
<td>Activation of monocytes</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Induction of megakaryocyte</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Eosinophil colony formation</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stimulation of eosinophil degranulation</td>
<td>-</td>
<td>-</td>
<td>+ (M)</td>
</tr>
<tr>
<td>Stimulation of histamine release</td>
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<td>(H)</td>
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(M) and (H) indicate activities described only in mouse or human cells, respectively.

are similar to the low-affinity site on hematopoietic cells. Two lines of evidence indicated that AIC2A is a binding component of a high-affinity mIL-3 receptor, ie, the high-affinity mIL-3 receptor purified by using biotin–mIL-3 contained the AIC2A protein and an AIC2A-specific monoclonal antibody (MoAb) partially inhibited the high-affinity binding. Based on these results, Hara and Miyajima cloned a cDNA (SUT-1) that confers a high-affinity mIL-3 binding in cooperation with AIC2A. The cDNA encodes a protein of 396 amino acid residues, which is a member of the type I cytokine receptor superfamily (Fig 1). The mature protein is about 70 Kd and has a capacity to bind mIL-3 by itself with low affinity (kd = 40 nmol/L). Coexpression of the SUT-1 and AIC2A proteins exhibits a high-affinity mIL-3 receptor (kd = 300 pmol/L). Chemical cross-linking experiments showed that the 120- to 140- and 70-kd proteins were cross-linked with mIL-3, identical with the cross-linking pattern observed in the hematopoietic cells. Moreover, the high-affinity mIL-3 receptor reconstituted in CTLL2 cells transmitted a growth signal in response to mIL-3, indicating that the high-affinity mIL-3 receptor is functional. Thus, SUT-1 and AIC2A are now referred to as the α subunit (mIL-3Rα) and the β subunit (βRIL-3) of the mIL-3 receptor, respectively (Fig 1).

### AIC2B AND A HUMAN AIC2 HOMOLOGUE (KH97) DO NOT BIND ANY CYTOKINE

Expression cloning using the anti-Aic2 antibody led to the identification of the second AIC2 cDNA, AIC2B, that encodes a protein of 896 amino acid residues with 91% identity to AIC2A. Interestingly, however, the AIC2B protein does not bind mIL-3 or any other cytokines including mouse GM-CSF (mGM-CSF) and mouse IL-5 (mIL-5). The structure of AIC2B is basically the same as βRIL-3 (Fig 1) and both proteins are coexpressed in various hematopoietic cells.

Using the mouse βRIL-3 cDNA as a probe, a human homologue of the βRIL-3 gene was cloned. The overall structure of the cloned cDNA, which was termed KH97, is almost the same as either the AIC2A or AIC2B proteins and shows about 56% identity with the mouse AIC2 proteins. KH97 has two repeats of the conserved motif of the cytokine receptor family and no consensus sequence for signaling molecules (Fig 1). Like AIC2B, KH97 does not bind any cytokine including human IL-3 (hIL-3), GM-CSF (hGM-CSF), and IL-5 (hIL-5). Despite extensive search for a cDNA encoding an hIL-3 binding protein using the mouse AIC2A cDNA probe, no such cDNA was found. Moreover, only one type of AIC2 homologous gene (KH97) was found in a human genomic library. These results raised two possibilities. First, the IL-3 binding protein has diverged extensively during evolution as IL-3 diverged extensively between mice.
and humans. The second possibility, which has proven to be the case, is that KH97 is a component of the hIL-3 receptor but does not bind hIL-3 by itself.

**RECONSTITUTION OF THE HUMAN HIGH-AFFINITY GM-CSF AND IL-3 RECEPTORS WITH THE COMMON β SUBUNIT, KH97**

Many similarities have been recognized among the IL-3, IL-5, and GM-CSF receptors. Chemical cross-linking experiments show two binding components for hIL-3, hGM-CSF, and hIL-5 receptors, a high molecular weight component of 120 Kd, regardless of the ligand, and a low molecular weight component of 60 to 80 Kd, depending on the cytokine. A human cell line TF-1 exhibits all three receptors and IL-1 coordinately upregulates the high-affinity receptors for hIL-3, hGM-CSF, and hIL-5. Moreover, the upregulation of the high-affinity receptors parallels the increase of the 120-Kd component of the three receptors in a similar manner. Interestingly, it is well established that the high-affinity hGM-CSF binding is competed by hIL-3 and vise versa as described above. Likewise, the hIL-5 binding is also competed by either hIL-3 or hGM-CSF on human eosinophils and basophils. This phenomenon known as cross-competition has been reported only in the human receptors but not in the mouse receptors. In addition, GM-CSF and IL-3 induce tyrosine phosphorylation of similar proteins and they have many overlapping biologic activities. These observations suggested the possibility that the IL-3, IL-5, and GM-CSF receptors may share the high molecular weight component.

Gearing et al isolated a cDNA encoding the 80-Kd GM-CSF receptor from a placenta cDNA library by expression cloning. The cDNA encodes a protein of 400 amino acids and has the common motif of the cytokine receptor superfamily in the extracellular domain. The cloned hGM-CSF receptor binds hGM-CSF with only low affinity when expressed on COS cells. All these results led Hayashida et al to test the possibility that a high-affinity hGM-CSF receptor consists of the cloned low-affinity hGM-CSF receptor and KH97. This was in fact the case: coexpression of the 80-Kd hGM-CSF receptor with KH97 formed a high-affinity hGM-CSF receptor. Cross-linking experiments showed that two proteins of 80 and 120 Kd were cross-linked. Although the KH97 protein by itself has no binding capability for hGM-CSF, it is cross-linked with GM-CSF when the high-affinity receptor is formed. Thus, the 80-Kd GM-CSF receptor and KH97 are now referred to as the α subunit (GMrα) and the β subunit of the GM-CSF receptor.

The model predicts that the β subunit (KH97) of the hGM-CSF receptor is also a component of the hIL-3 receptor. In contrast to the mIL-3 receptor, no low-affinity hIL-3 binding site was found on murine cells, suggesting the possibility that the putative hIL-3Ra has no hIL-3 binding capacity by itself or binds it with very low affinity. If this were the case, direct expression cloning would be difficult. Therefore, Kitamura et al screened for hIL-3 binding of COS cells cotransfected with pools of the cDNA library and the KH97 cDNA. The cDNA cloned by this procedure encodes a protein of 378 amino acids and the extracellular domain has the common motif of the cytokine receptor family. The mature protein is about 70 Kd and the structure is similar to mIL-3Ra, but they show only 30% identity at the amino acid level. The overall structure of hIL-3Ra and hGMrα are similar and there is a short stretch of conserved amino acid sequence, Arg-Leu-Phe-Pro (RLFP), in the cytoplasmic domains. Whereas COS7 cells transfected with the hIL-3Ra cDNA alone bound hIL-3 with extremely low affinity (kd ~100 nmol/L), cotransfection of the hIL-3Ra cDNA and the KH97 cDNA reconstituted a high-affinity hIL-3 receptor. The dissociation constant was about 100 pmol/L, which is the same as that of the high-affinity receptor on hematopoietic cells. Chemical cross-linking of 125I-hIL-3 showed that both subunits were cross-linked only when the high-affinity receptor was formed. Thus, it is clear that the KH97 protein is used for both hIL-3 and hGM-CSF receptors. Therefore, KH97 is now referred to as the common β subunit or βk. The lack of a low-affinity binding site for hIL-3 in human hematopoietic cells is caused by the absence of the AIC2A homologue in the human and also by the extremely low-affinity binding of hIL-3Ra to hIL-3.

Because the IL-3 and GM-CSF receptors share the same component for high-affinity binding, the cross-competition of binding between hGM-CSF and hIL-3 is likely caused by the competition for a limiting number of βk by different α subunits. This possibility was experimentally verified by NIH3T3 cells stably transfected with the hGMrα, hIL-3Ra, and βk cDNAs. The transfectants exhibited both the high-affinity hIL-3 and hGM-CSF binding sites and cross-competition of binding between hIL-3 and hGM-CSF was clearly shown. The extent of cross-competition varied depending on the expression levels of the subunits. If the expression level of βk exceeded those of the α subunits, no cross-competition was observed. These results indicate that the cross-competition of binding between hIL-3 and hGM-CSF occurs by competition for βk between different α subunits.

**AIC2B IS THE MOUSE COMMON β SUBUNIT**

Because the IL-2–dependent mouse T-cell line CTLL2 does not express endogenous mouse IL-3 and GM-CSF receptor subunits, it provides a convenient assay system to test the function of these receptors. The hGMrα alone expressed in CTLL2 does not stimulate cell proliferation in response to hGM-CSF. Coexpression of hGMrα and human βk confers high-affinity hGM-CSF binding and proliferation in response to hGM-CSF. Interestingly, although CTLL2 cells coexpressing hGMrα and mouse AIC2B bind hGM-CSF with only low affinity, they proliferate in response to hGM-CSF, suggesting that AIC2B is the mouse β subunit for GM-CSF. However, coexpression of hGMrα and the mouse βIL3 subunit (AIC2A) does not form a functional GM-CSF receptor. Park et al recently isolated a cDNA encoding the mGMrα by expression. The homology between mouse and human GMrα subunits is only 35% identical at the amino acid level. mGMrα reconstitutes a high-affinity mGM-CSF receptor with AIC2B, but not with AIC2A, consistent with the results obtained with hGMrα and mouse β subunits.
Similar to the hGM-CSF receptor, the high-affinity hIL-3 receptor transmits growth signals in CTLL2 cells, whereas hIL-3Ra alone is unable to stimulate proliferation even in the presence of high concentrations of hIL-3. Coexpression of hIL-3Ra with either AIC2B or \( \beta_{h3} \) does not change the affinity significantly. Interestingly, however, CTLL2 cells that coexpress hIL-3Ra with AIC2B but not with \( \beta_{h3} \) proliferated in the presence of high concentrations of hIL-3. This is a rather surprising result, but suggests that AIC2B is the mouse counterpart of \( \beta_{h3} \). Cloning of the mIL-3Ra and reconstitution of high-affinity IL-3 receptors showed that mIL-3Ra forms high-affinity IL-3 receptors with either AIC2A (\( \beta_{h3} \)) or AIC2B (\( \beta_{h} \)). A high-affinity mIL-3 receptor with either AIC2A or AIC2B transmits growth signals in CTLL2 in response to mIL-3. There is no clear difference in either the binding properties or signal transduction between the two high-affinity mIL-3 receptors. Thus, the mIL-3R has two distinct functional \( \beta \) subunits, ie, one (AIC2A, or \( \beta_{h3} \)) is IL-3 specific and the other (AIC2B, or \( \beta_{h} \)) is common between mIL-3 and mGM-CSF receptors.

**IL-5 RECEPTOR**

Mouse IL-5 (mIL-5) binds to both high- and low-affinity receptors. Cross-linking shows two distinct binding components, 60 and 130 Kd. Takaki et al cloned a cDNA for the 60-Kd component by expression using an MoAb against AIC2A (\( \beta_{h3} \)) or AIC2B (\( \beta_{h} \)). A high-affinity mIL-3 receptor with either AIC2A or AIC2B transmits growth signals in CTLL2 in response to mIL-3. There is no clear difference in either the binding properties or signal transduction between the two high-affinity mIL-3 receptors. Thus, the mIL-3R has two distinct functional \( \beta \) subunits, ie, one (AIC2A, or \( \beta_{h3} \)) is IL-3 specific and the other (AIC2B, or \( \beta_{h} \)) is common between mIL-3 and mGM-CSF receptors.

Cross-competition was shown in COS cells transiently transfected with the hGMRa, hIL-5Ra, and the human \( \beta_{h} \) cDNAs. Whereas the cross-competition of binding between mGM-CSF and mIL-5 has not been reported, the model predicts that this may occur in mouse cells as well.

**EXPRESSION AND GENES OF THE RECEPTOR SUBUNITS**

Based on the model presented in Fig 1 and cellular response to cytokines, it is assumed that \( \beta_{h3} \) is expressed in various lineages of hematopoietic cells as well as the early multipotential progenitors. IL-3Ra is also expected to be widely distributed, including early progenitors and lineage committed cells. In contrast, IL-5Ra expression may be restricted to basophils, eosinophils, and some B cells. MoAbs against each receptor subunit as well as cDNA probes have been used to examine expression of the receptors in hematopoietic and nonhematopoietic cells. As anticipated, \( \beta_{h} \) and IL-3Ra are expressed in various lineages with myeloid markers such as CD13\(^+\), CD14\(^+\), CD15\(^+\), or CD33\(^+\) as well as early progenitors with CD34\(^+\). Neither \( \beta_{h} \) nor IL-3Ra is expressed in cells with the T-cell marker CD3, but some fraction of cells with the B-cell marker CD19 express both \( \beta_{h} \) and IL-3Ra. The GM-CSF receptor is also expressed in nonhematopoietic cells. Expression of these receptor subunits is affected by other cytokines. IL-1 and tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) induce upregulation of \( \beta_{h} \) in TF-1 cells. TNF-\( \alpha \) and interferon-\( \gamma \) (IFN-\( \gamma \)) also increase the \( \beta_{h} \) expression in CD34\(^+\) and monocytes, respectively. The transcription of the mouse \( \beta_{h3} \) and \( \beta_{h} \) genes is transiently increased in erythroid and myeloid progenitor cell lines by EPO.

Because expression of these hematopoietic cytokine receptors is generally restricted to hematopoietic cells, expression of these receptors must be regulated in a cell-type specific manner. Murine embryonic stem cells express neither \( \beta_{h3} \) nor \( \beta_{h} \) under a culture condition that maintains the totipotency. Dramatic upregulation of the two \( \beta \) subunit genes occurs at day 6 or 7 in a culture condition that allows differentiation to various lineages. Similarly, blastocysts at day 3 or 4 express no detectable \( \beta \) subunit mRNA and induction of the \( \beta \) subunit occurs after 7 to 9 days culture in vitro, which is consistent with the time of blood island formation. Thus, the temporal pattern of the \( \beta \) subunit expression may serve as an excellent marker for the commitment to the hematopoietic lineage. Surprisingly, in contrast to the \( \beta \) subunits, expression of both IL-3Ra and GMRa mRNA was detectable in the ES cells as well as day 3 or 4 blastocysts, yet their expression in differentiated cells is restricted to hematopoietic cells, suggesting the presence of a complicated regulatory mechanism for expression of these subunits. IL-5Ra was not detected in ES or day 3 or 4 blastocysts and the expression was detected in day 22 culture of the blastocysts. As the IL-5 receptor is expressed on early murine B cells, the late expression of IL-5Ra in embryoid bodies may correlate with the late wave of lymphoid development.

Like many other genes, expression of the cytokine receptor genes may be regulated mainly at the transcriptional level in a cell-type specific and temporal manner. While
information about the transcriptional control of the receptor expression is currently limited, it is an important aspect of understanding the regulation of hematopoiesis. In addition to the transcriptional control, the cytokine receptor expression appears to be regulated at the posttranscriptional levels as well. Alternative splicing often produces mRNA encoding a soluble external domain of a cytokine receptor. cDNAs encoding a soluble IL-5Ra or GMRa have been isolated. Because GMRa or IL-5Ra alone binds its ligand, soluble receptors are potential antagonists. In fact, soluble IL-5Ra was shown to inhibit IL-5-driven eosinophil proliferation. Interestingly, the major transcript of the IL-5Rα gene in both IL-60 eosinophilic cells and eosinophilic myelocytes grown from cord blood encodes a secreted form of IL-5Ra. However, various mouse IL-5 responsive cells as well as eosinophils from a patient with eosinophilia contained differing ratios of mRNAs for both the membrane-bound and the soluble form of IL-5Ra. Thus, it is possible that splicing of cytokine receptor mRNAs is regulated in a cell-type specific manner. Receptor variants with an alteration in the external domain or the cytoplasmic domain were also found for β1, IL-3Ra, hGMRa, and hIL-5Rα. Function of these alternative forms is currently unknown. In addition to splicing, other posttranscriptional mechanisms appear to be present. Evidence indicates that a posttranscriptional mechanism dominantly suppresses expression of β1 and β2 in some mouse hematopoietic cells.

The genes for the receptor subunits have been mapped. Two mouse β subunit genes are tightly linked on chromosome 15. They are located next to the IL-2 receptor β subunit gene as well as the sis proto-oncogene. This region corresponds to the human chromosome 22 and, in fact, the β2 gene was mapped to 22q11-22q13. While there seems to be only one β subunit gene in the human, the exon-intron structures of the two mouse β subunit genes are almost identical and the 5′ flanking and intron sequences are also well conserved, suggesting that the two β subunit genes arose in the mouse by gene duplication after divergence between mice and humans. High conservation of the 5′ flanking sequences of the two mouse β subunit genes is consistent with the coexpression of both β subunits in various hematopoietic cells. The hGMRa and hIL-3Ra cDNAs are present on the pseudoautosomal region of the X and Y chromosomes. However, the hIL-5Rα gene is on the chromosome 3p24-3p26. In the mouse, all three α subunit genes are present on different chromosomes, i.e., mGMRa, mIL-3Rα (I. Miyajima, L. Levitt, N. Jenkins, A. Miyajima: in preparation), and mIL-5Rα are on chromosome 19, 14, and 6, respectively. This contrasts with the fact that GM-CSF, IL-3, and IL-5 genes are tightly linked on human chromosome 5 and mouse chromosome 11.2

INTERACTIONS BETWEEN CYTOKINES AND THEIR RECEPTORS

GM-CSF, IL-3, and IL-5 are cytokines with four α-helices (Fig 2). Although there is no significant amino acid sequence homology among the three cytokines, their gross tertorially structures are similar. The structure of GM-CSF has been extensively studied physically by crystallography, as well as genetically using numerous mutations. GM-CSF is species specific and hGM-CSF does not bind to mGMRa and vice versa. This species specificity proved to be useful for the analysis of the interaction between GM-CSF and its receptor. A series of hybrid GM-CSF between mouse and human GM-CSF was used to identify the sequence involved in the interaction with the receptor. The hybrid GM-CSF that has the N-terminal α helix derived from mGM-CSF and the rest of the sequence derived from hGM-CSF binds with high affinity to the composite receptor consisting with hGMRa and mouse β2, indicating that the N-terminal α helix is recognized by the β2 subunit (Fig 2). Because the β2 subunit is shared between GM-CSF, IL-3, and IL-5, this result suggests the possibility that β2 recognizes the N-terminal α helices of all these α subunits. Indeed, a hybrid cytokine between mIL-5 and hGM-CSF, which has the N-terminal α helix derived from mIL-5 and the rest derived from hGM-CSF, binds with high affinity to the composite receptor between hGMRa and mouse β2. The N-terminal α helix of mIL-3 is also recognized by mouse β2.

Amino acid sequence comparison between N-terminal α helices of mouse GM-CSF, IL-3, and IL-5 receptors shows the presence of two identical amino acid residues, Lys and Glu, in 14 amino acid residues in the α helices. Substitution of the Glu21 residue of mGM-CSF with Ala abrogates binding to the β2 subunit and thereby eliminates the high-affinity binding. Interestingly, an acidic amino acid (Glu or Asp) is conserved among IL-2, IL-3, IL-4, IL-5, and IL-7 in the position corresponding to Glu21 of mGM-CSF. The acidic residue Asp34 in the N-terminal α helix of mIL-2 is critical for binding to mIL-2 receptor β subunit. High conservation of the amino acid sequences between mouse β2 and β1,3,4 allowed identification of critical amino acid residues of β1,3 for IL-3 binding. Those amino acid residues essential for IL-3 binding are located between the two barrel-like structures in the C-terminal cytokine receptor motif of β1,3. Interestingly, the region of the IL-2 receptor β subunit that interacts with the critical Asp34 residue of mIL-2 was mapped to the region between the two barrel-like structures, which corresponds to the region mapped on β1,3 for the IL-3 binding. These results are consistent with the model proposed by Bazan and also with the co-crystal structure of growth hormone and the growth hormone receptor, another member of the cytokine receptor family.

REQUIREMENT OF CYTOPLASTIC DOMAINS OF THE α AND β SUBUNITS FOR SIGNAL TRANSDUCTION

Binding of a cytokine to the receptor is not sufficient to transduce signals. CTLL2 cells transfected with either mIL-3Rα or β1,3 cDNA bind mIL-3 with low affinity but do not proliferate in the presence of mIL-3, whereas CTLL2 expressing both subunits proliferate, indicating that the high-affinity receptor consisting of both α and β subunits is required for signaling. On the other hand, high-affinity binding of a cytokine to the receptor is not necessary in certain cases, e.g., a composite receptor between the hGM-CSFRα and mouse β2 binds hGM-CSF with only low affinity but
transduces signals in response to hGM-CSF. A mutant mGM-CSF with a substitution of Glu21 with Ala abrogates the high-affinity binding to the GM-CSFR, but is still capable of transducing growth signal in the presence of high concentrations of mGM-CSF. These results indicate that binding of a cytokine to its α subunit leads to a tight association with the β subunit to transduce signals and the association between the α and β subunits seems to be the most crucial step for signal transduction. Even if the cytokine does not directly contact the β subunit it still can induce signals unless the interaction between the α and β subunits is impaired.

Thus, the β subunits are essential not only for high-affinity binding of a cytokine, but also for signal transduction. Cytoplasmic deletions of the human β, defined critical regions for signal transduction. The membrane-proximal portion of β, is essential for mitogenic signaling. The GM-CSF receptor with the β517 mutation, which is truncated at amino acid 517 and retains only about 60 amino acid residues from the transmembrane domain, is able to induce mitogenic signals in response to GM-CSF. Interestingly, the membrane proximal cytoplasmic regions of other cytokine receptors including IL-2 receptor β subunit, IL-4 receptor, EPO receptor, G-CSF receptor, and gp130 also play an important role for mitogenic signaling and in fact there are some sequence similarities among these regions.

Unlike the IL-6 receptor in which the cytoplasmic domain of the IL-6 binding component is dispensable for signal transduction, the cytoplasmic domains of the α subunits of the GM-CSF/IL-3/IL-5 receptors are required for signal transduction. Deletion of the cytoplasmic domain of α subunits does not affect the binding affinity, but abrogates signaling, suggesting that the α subunits are not only required for binding of the cytokine, but also are important for signal transduction and the α subunits may be responsible for induction of cytokine-specific signals. If this is the case, variants of GMRα and IL-5Rα with a different C-terminal region, which are produced by alternatively spliced mRNAs, may have a specific function.

Requirement of cytoplasmic domains of two subunits of the cytokine receptors appears to be a general principle. The functional high-affinity IL-2 receptor consists of α, β, and γ subunits. The β and γ subunits are members of the cytokine receptor superfamily. Whereas the α subunit is dispensable for signal transduction, cytoplasmic domains of the β and γ subunits are essential. IL-6 binding to the IL-6 receptor induces dimerization of gp130. G-CSF receptor also forms a homodimer. LIF, OSM, and CNTF receptors requires both gp130 and LIF binding protein, both of which are members of the cytokine receptor family. Interestingly, chimeric receptors between different members of this family are functional. Chimeric receptors consisting of the G-CSF receptor extracellular domain and the cytoplasmic domain of either gp130 or LIFBP induce signals in response to G-CSF. Similarly, chimeric receptors consisting of the cytoplasmic domain of β1 and the extracellular domain of either IL-2Rβ, IL-4R, or EPO receptor are functional and induces growth signal in response to either IL-2, IL-4, or EPO, respectively, in appropriate cells. Likewise, the
chimeric receptor consisting of the extracellular GM-CSFRα and the intracellular EPO receptor was functional and stimulated proliferation and induction of glycoporphin, an erythroid-specific molecule, in response to GM-CSF in BaF3 cells.98 In contrast, whereas IL-2 induces not only proliferation but also upregulation of IL-2Rα through the IL-2 receptor, IL-2 failed to induce IL-2Rα expression through the chimeric receptor consisting of the IL-2Rβ extracellular domain and the β1L3 intracellular domain.97 These results suggest that cytokine-dependent proliferation can be achieved by different combinations of the cytoplasmic domains among the members of the cytokine receptors and functions specific to a cytokine such as the induction of IL-2Rα by IL-2 may require a specific combination of the cytoplasmic domains.

TYROSINE PHOSPHORYLATION

Study of the biochemical interactions involved in signaling from the GM-CSF/IL-3/IL-5 receptors has suggested that pathways important for signal transduction from growth factor receptors,99 specifically the extensively studied receptors for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), also function in the GM-CSF/IL-3 system. Similar to EGF or PDGF, one of the earliest events after ligand binding is induction of protein tyrosine phosphorylation.

Although none of the subunits in GM-CSF/IL-3/IL-5 receptors has an intrinsic tyrosine kinase, several lines of evidence have indicated involvement of a tyrosine kinase in signaling pathways of IL-3/GM-CSF. Oncogenes encoding tyrosine kinase activity abrogate the factor requirement of factor-dependent cells.100-103 Moreover, tyrosine kinase inhibitors inhibit proliferative response to cytokines such as IL-3,106 while tyrosine phosphatase inhibitors sustain the short-term growth of a GM-CSF- or IL-3-dependent cell line.10,107 Of particular importance is that IL-3/GM-CSF induces tyrosine phosphorylation rapidly, even when cells are stimulated at 4°C,108 suggesting the possibility that the kinase and its initial substrates are closely associated with the receptor before ligand stimulation.

While several hematopoietic tyrosine kinases, including Lyn,109 Fyn,110,111 and Fps,112 are stimulated by GM-CSF or IL-3 in hematopoietic cells, the GM-CSF receptor ectopically expressed in NIH3T3 fibroblast cell line that lacks the expression of these hematopoietic cell-specific kinases undergo tyrosine phosphorylation in response to GM-CSF.113-116 Likewise, IL-2 binding to the IL-2 receptor results in activation of the Lck tyrosine kinase in T cells, but the IL-2 receptor is equally functional in BaF3 cells that lack Lck expression.111 These observations suggest that multiple nonreceptor tyrosine kinases can be activated by a cytokine receptor. Involvement of the Jak2 tyrosine kinase in cytokine receptor signaling has recently been found.117-119 Jak2 is a member of the tyrosine kinase subfamily including Tyk2 that has been implicated in the IFN-α receptor signaling.120-122 Stimulation with IL-3 or EPO results in the rapid tyrosine phosphorylation of Jak2 and activates its in vitro kinase activity.117,118 Furthermore, phosphorylation and activation of kinase activity correlate with the induction of mitogenesis. Jak2 physically associates with a membrane proximal region of the cytoplasmic domain of the EPO receptor that is required for mitogenesis. Jak2 has also been involved in growth hormone receptor signaling.119 As Tyk2, another member of the Jak family, is implicated in phosphorylation of the cytoplasmic transcription factor ISGF-3 which translocates to the nucleus on tyrosine phosphorylation and stimulates IFN-α specific gene expression,120,121 Jak2 may also phosphorylate a putative ISGF3-like molecule and regulate gene expression (Fig 3). However, because many cytokines with varying biologic activities all stimulate the ubiquitously expressed Jak2, biologic specificity must occur at levels other than Jak2 activation. Cytoplasmic domains of the cytokine receptors may contribute to the specificity of substrates.

Many tyrosine phosphorylated proteins have been described in both human10,12,108,122,123 and mouse124-126 cells stimulated with IL-3 or GM-CSF. As expected, IL-3 and GM-CSF induce identical phosphorylations, in the same cell line,10,12 that are different from those induced by ligands with structurally distinct receptors.127,128 Interestingly, IL-2 or EPO stimulates phosphorylation of similar proteins as IL-3 or GM-CSF,129-131 notably a 90- to 100-Kd molecule, which suggests an early convergence of signaling pathways. A few of the GM-CSF/IL-3-induced tyrosine phosphorylated substrates have been identified, which include: the β5 subunit itself,77,126,132-133 Vav,134 Fps,112 Raf,135,136 Shc, and MAP kinase (MAPK).137-140 Significantly, only a subset of these phosphorylations, those of the β5 subunit, Shc, and the 90- to 100-Kd molecule, occur when cells are stimulated at 4°C, whereas Vav and MAPK phosphorylation and Raf kinase activation occur only when cells are treated at 37°C. This observation suggests, perhaps, that the tyrosine kinase responsible for these phosphorylations is closely associated with the β5 subunit, and certain substrates, before ligand stimulation. The other substrates, Raf, MAPK, and possibly Vav, function further downstream of the receptor in the signaling cascade. However, it is important to note that more than one tyrosine kinase functions during signal transduction. As described below, deletion analysis of the β5 subunit suggests that the signal responsible for the major tyrosine phosphorylations induced by GM-CSF can be separated from tyrosine phosphorylation upstream of c-myc induction.77,140 Also, analogous to the model proposed for the T-cell antigen receptor (TCR), different tyrosine kinases may act sequentially.141 It is also important to note that although most attention has been focused on tyrosine kinases, the action of tyrosine phosphatases is also essential. For example, the Src-type kinases are negatively controlled by phosphorylation at a regulatory tyrosine and removal of the phosphorylation at this site is required for kinase activation.142 The lymphocyte-specific, tyrosine phosphatase CD45 functions in TCR signaling by dephosphorylating the regulatory tyrosine in Lck.143 Prior action of a tyrosine phosphatase in tyrosine kinase activation has also been suggested in other receptor systems,144 although the identity of the phosphatase has not been established. One phosphatase, PTP1C145,146 among the...
Fig 3. A model of signaling pathways from the GM-CSF/IL-3 receptors. CK and PTK stand for cytokines and protein tyrosine kinases, respectively. Although several tyrosine kinases are drawn for diagrammatic reasons, their number and identity have yet to be established. Broken lines indicate hypothetical pathways.

large family of tyrosine-specific phosphatases, is especially intriguing for cytokine receptor signaling because it is expressed predominantly in hematopoietic cells.

One of the major tyrosine phosphorylated proteins induced by IL-3/GM-CSF stimulation is the β subunit, although its significance is unclear. Deletion analysis of βc suggests that the tyrosine phosphorylation site is dispensable for mitogenic signaling; however, it might be important in transducing alternate signals such as those for cellular differentiation or activation. Tyrosine phosphorylation of the receptor might also be implicated in downregulation of the receptor as tyrosine phosphorylation of the mIL-3 receptor increases its susceptibility to proteolysis. In contrast, the role of tyrosine phosphorylation of receptors with an intrinsic tyrosine kinase has been better defined; receptor autophosphorylation generates sites that bind signaling molecules containing SH2 (Src homology 2) domains. SH2 domains consist of about 100 amino acid residues, which were first identified in Src-like kinases, that mediate the interaction with phosphotyrosine. Signaling molecules that possess SH2 domains include Vav, Shc, as well as the p85 subunit of phosphatidylinositol 3 kinase (PI3 kinase), phospholipase Cγ (PLCγ), Ras GTPase activating protein (GAP), and Grb2. Binding specificity of different SH2 domains is determined by the amino acid sequence surrounding the phosphorylated tyrosine. While no consensus sequence for SH2 binding motif has been found in the cytokine receptors, SH2 containing proteins appear to be involved in cytokine signaling, eg, stimulation with IL-3 or GM-CSF results in association of tyrosine phosphorylated protein(s) with SH2-containing proteins. In addition, IL-3/GM-CSF induce tyrosine phosphorylation of SH2-containing proteins such as Shc or Vav, suggesting the possibility that they associate with cytokine receptors via an intermediate, adaptor protein as has been shown for the insulin and B-cell antigen receptors, and postulated for the IL-4 receptor.

SIGNALING PATHWAYS FROM HEMATOPOIETIC CYTOKINE RECEPTORS

Stimulation of a growth factor receptor with an intrinsic tyrosine kinase induces phosphorylation of proteins that lead to activation of various signaling molecules. Although Ras itself is not tyrosine phosphorylated, tyrosine kinases have been implicated in the Ras activation pathway both in cytokine and growth factor receptor systems. Ras is a small molecular weight, membrane-associated, GTP binding protein that is involved in signaling from various growth factor receptors and cycles between GTP-bound active and GDP-bound inactive states in response to extracellular signals. This switching can be controlled through activation of its intrinsic GTPase activity, by proteins such as GAP, or by stimulation of Ras GDP/GTP nucleotide exchange factors such as Sos. However, recent excitement in study of Ras regulation has centered on the discovery that Shc and Grb2 links activated intrinsic tyrosine kinase receptors to Sos and on the demonstration that Vav, a hematopoietic-specific molecule, also has nucleotide exchange activity. She and Grb2 are small proteins consisting almost entirely of SH2 and SH3 (Src homology 3) domains. Shc and Grb2 and, in some systems, Grb2 alone, associates with Sos and the complex binds to activated growth factor receptors through the interaction of Shc or Grb2 SH2 domains with receptor phosphotyrosines. Thus, the overall cellular level of Sos nucleotide exchange activity does not change upon growth factor stimulation; instead, Sos is controlled by being brought into proximity of its substrate, Ras. Although the linkage between the cytokine receptors and activation of Ras is still unknown, IL-3/GM-CSF induce tyrosine phosphorylation of Shc and thus, like the growth
factor systems, Shc may couple the receptor to the Ras GTP exchange factor. Like Shc and Grb2, Vav is an SH2- and SH3-containing molecule, but unlike Shc and Grb2, Vav is expressed only in hematopoietic cells. In addition to its SH2 and SH3 domains, Vav has a domain with Ras GDP/GTP nucleotide exchange activity. Tyrosine phosphorylation of Vav in vitro by Lck activates its nucleotide exchange activity and almost all of the exchange activity in anti-CD3 activated T cells is attributable to Vav. Because IL-3/GM-CSF also induce tyrosine phosphorylation of Vav, Vav may also link these receptors to Ras (Fig 3). The presence of this additional Ras regulatory pathway in hematopoietic cells may also provide a basis for the ability of PDGFR mutants, which are incapable of activating Ras in nonhematopoietic cell lines, to activate Ras in an IL-3-dependent, pro B-cell. It would be interesting, regardless, to investigate the contribution of the Shc and Vav pathways to Ras function in hematopoietic cells.

Downstream of Ras is a serine/threonine kinase, Raf, which regulates another serine/threonine kinase MAP kinase (MAPK) through activation of MAPK kinase, a protein-tyrokinase/threonine kinase. Activation of MAPK leads to induction of c-fos/c-jun. This signaling cascade, consisting of Ras-GTP elevation, phosphorylation, and activation of Raf and MAPK, and induction of c-fos and c-jun, appears to be stimulated by GM-CSF/IL-3/5125,156,160 as well as other cytokines such as IL-2164,165 and EPO.166,167 Interestingly, analysis of the cytoplasmic deletion mutants of the human β subunit showed that the same cytoplasmic region is responsible for all these biochemical events, consistent with the model that these events are induced as a cascade as described for other growth factor systems (Fig 3).

As the pathway that leads to induction of c-fos from Ras appears to be common to the cytokine receptors and growth factor receptors, a growth factor receptor with intrinsic tyrosine kinase activity may replace functions of a cytokine in hematopoietic cells and conversely, the cytokine function may function in nonhematopoietic cells such as fibroblasts. In fact, hematopoietic cells transfected with the EGF receptor activate Ras, induce c-fos, and stimulate proliferation in response to EGF.164,168,169 Conversely, the high-affinity GM-CSF receptor ectopically expressed in NIH3T3 fibroblasts stimulates tyrosine phosphorylation of the β subunit as well as other cellular proteins, induces nuclear proto-oncogenes, c-myc, c-fos, and c-jun, and stimulates proliferation in response to GM-CSF.113,114,116 Moreover, some NIH3T3 transfectants expressing the GM-CSF receptor were transformed by GM-CSF.115,116 These results indicate that the pathway involving activation of Ras to induction of c-fos is common to the growth factor receptors and hematopoietic cytokine receptors, although the precise link between the ectopically expressed receptor and activation of Ras is not clear.

Expression of c-myc has been implicated in cell proliferation in various systems. Deletion analysis of β also indicates a close correlation between c-myc induction and cell proliferation. While cells expressing the cytoplasmic deletion mutant of β, β17, which retains only about 60 amino acid residues from the transmembrane domain, are unable to induce major tyrosine phosphorylation by GM-CSF, the cells still proliferated in response to GM-CSF and the GM-CSF-induced proliferation is sensitive to herbimycin, a tyrosine kinase inhibitor. Moreover, GM-CSF induces c-myc in cells with β17 and the induction is also sensitive to herbimycin (Fig 3). Taken together, c-myc expression appears to be important for proliferation induced by GM-CSF/IL-3. As c-myc is induced by the ectopically expressed GM-CSF receptor in NIH3T3 cells,113,114 the signaling pathway to c-myc induction in fibroblast can be linked to the GM-CSF receptor, although the mechanism of c-myc induction by GM-CSF/IL-3 is still unknown.

Proto-oncogene pim-1 encodes a serine/threonine kinase that is expressed specifically in hematopoietic cells. Pim-1 is rapidly induced by various cytokines including IL-2, IL-3, and GM-CSF and cooperates with Myc in lymphomagenesis. Proliferation of mast cells derived from Pim-1-deficient mice in response to IL-3 is slower than that of normal mice, indicating that Pim-1 has some role in IL-3 response but is not absolutely required. Interestingly, while the same region of β, is responsible for induction of both c-myc and pim-1 by GM-CSF, cooperation of pim-1 and c-myc suggests that they may act in different pathways (Fig 3). As pim-1 is specific for hematopoietic cells, it may have a role unique to hematopoietic cells.

Activation of various growth factors leads to tyrosine phosphorylation of PLCγ. Activated PLCγ hydrolyzes phosphatidylinositol (PI) to generate diacylglycerol (DG) and inositol triphosphates which, in turn, activate protein kinase C (PKC) and mobilize calcium, respectively. In contrast, although IL-3/GM-CSF activate PKC, PI hydrolysis appears not to be involved in the DG formation by IL-3/GM-CSF. Instead, evidence has been presented that IL-3/GM-CSF induce the DG formation through hydrolysis of phosphatidylycholine (PC), although the linkage between the cytokine receptors and PC-PLC is not clear yet. A recent report suggests that the PI3 kinase product activates PKCζ, one of the PKC isozymes. As IL-3/GM-CSF increase PI3 kinase activity in antiphosphotyrosine immunoprecipitates, IL-3/GM-CSF may activate PKCζ by inducing association of PI3 kinase with tyrosine phosphorylated proteins (Fig 3).

CONCLUSION

Molecular cloning of the cytokine receptor genes has shown that functional receptors consist of more than one subunit. The finding that the GM-CSF, IL-3, and IL-5 receptors share the common β subunit with signaling function provides a basis for common biologic functions displayed by GM-CSF, IL-3, and IL-5. Likewise, IL-6, LIF, OSM, CNTF, and IL-11 receptors share the common subunit, gp130, which is essential for their signal transduction. While their receptor structures have not been elucidated, IL-4 and IL-13 exhibit a number of similar functions and IL-13 competes with IL-4 for binding in some cells, suggesting that they may share a common receptor component. X-linked severe combined immune deficiency (SCID), which is caused by a mutation in the IL-2 receptor γ subunit
gene,\(^1\) shows more severe phenotype than IL-2-deficient immune deficiency, suggesting that the \(\gamma\) subunit may be involved in other cytokine receptors. Thus, it appears to be general among the cytokine receptors that a subset of cytokine receptors share the common subunit that is essential for signal transduction. The shared receptor subunits provide an explanation for why distinct cytokines exhibit similar functions on the same target cell. On the other hand, while there are only one or two types of receptors for each cytokine, each cytokine exhibits pleiotropic functions depending on cell type. Therefore, the pleiotropic function of a cytokine must be explained by cellular signaling differences and future studies need to address how the cell surface receptor is coupled to the diverse intracellular signaling pathways and how each signaling pathway is linked to diverse functions in each cell type.

ACKNOWLEDGMENT

We thank Gary Burget for preparation of the figures.

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