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Cytokine and Growth Factor Reviews 12 (2001) 19–25

Cytokine & Growth Factor Reviews

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Mini Review

Specificity in cytokine signal transduction: lessons learned from the IL-3/IL-5/GM-CSF receptor family

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Abstract

Cytokines mediate the transduction of proliferative, differentiation and survival signals in the hematopoietic system. Although the cytokine family is large and diverse, many different cytokines display broadly overlapping functions. This can be explained by the fact that cytokine receptors often share multiple subunits. Specificity in signal transduction can however be achieved through several mechanisms. This review focuses on how signal specificity can be achieved within the IL-3, IL-5 and GM-CSF receptor family. This is discussed in terms of receptor expression, recent advances in our understanding of intracellular signalling components, and analysis of null mutant knock-out mice. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Cytokine; Receptor; Specificity; Signalling

1. Introduction

Cells of the hematopoietic system communicate utilising a large family of polypeptide growth factors termed cytokines. These proteins mediate the communication of proliferative, differentiation and activation signals to target cells. Cytokines are pleiotropic such that a specific cytokine may act on many different cell types, eliciting a wide range of biological responses, depending on the target cell. Furthermore, cytokines are also redundant, since distinct cytokines can act on a single target cell to elicit the same response. Understanding the molecular structure of cytokine receptors has provided an explanation for the functional redundancy of cytokines (Fig. 1). Many cytokine receptors consist of multiple subunits, one of which is cytokine specific [1]. Other subunits mediate cytokine-activated signal transduction pathways and can be shared between several cytokine receptors. Why have so many cytokines developed if they have overlapping functions? One could imagine that a complex system with high redundancy has developed for the purpose of stability. Indeed, many cytokines that have been shown to be essential for certain specific processes in vitro, fail to show severe phenotypical defects in null-mutant mice (see below). This raises the question of how, in such a complex and redundant system, specificity is achieved? In this review we will discuss some of the mechanisms underlying cytokine specificity in view of the IL-3/IL-5/GM-CSF receptor family.

2. Structural organisation of the IL-3, IL-5, GM-CSF receptor family

The receptors for IL-5, IL-3 and GM-CSF each consist of a cytokine specific α-chain, which binds ligand with low affinity [2,3], and a common β-chain (βc), which is shared among all the receptors of this family. The βc by itself does not bind ligand, but forms a high affinity cytokine receptor only upon association with the α-chain. In the mouse, a second β-chain has been identified which associates exclusively with the IL-3 α-chain (IL-3Rα), hence its name β_{IL-3} . Although, this second βc_{IL-3} was thought to be the result of a gene duplication after the separation between human and mouse, recently we have identified remnants of a

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PII: S1359-6101(00)00019-8

former human $\beta_{\text{IL-3}}$ whose functionality has apparently been lost later in evolution [4].

Many factors may contribute to specificity in signalling between the IL-3, IL-5 and GM-CSF receptors. These receptors are differentially expressed throughout the hematopoietic system [5]. For example, the IL-5 receptor is expressed exclusively on eosinophils, basophils and mouse B cell precursors as well as mature B1 cells. Stimulation with IL-5 will thus result in the activation of a distinct subset of cells bearing the respective cytokine receptor. However, many myeloid and lymphoid progenitors may express two or more of these receptors concomitantly.

Many receptor-initiated signals are amplified as they are transduced. Subtle differences in signalling kinetics at the receptor level, or receptor expression levels may result in marked differences in the activation of downstream signalling components. For example, Rossi et al. have demonstrated that the lineage commitment of a hematopoietic precursor cell line can be controlled by the level of PKC activity [6]. While the absence of PKC activity maintained cells in an undifferentiated state, low PKC activity induced differentiation of cells towards a myelomonocytic phenotype. High PKC activity however, favoured eosinophilic differentiation. Moreover, it has been reported that high concentrations of IL-3 promote self-renewal of a murine multipotential hematopoietic cell line, whereas low concentrations of IL-3 in combination with GM-CSF or erythropoietin result in differentiation towards granulocytes, macrophages or erythroid cells [7,8]. There is also evidence for specific intracellular signalling from each individual receptor within one target cell. IL-5 has been reported to be essential for the differentiation of early $CD34^{+}$ progenitor cells into eosinophil colonies in vitro, whereas IL-3 does not support eosinophil colony formation, even though these early progenitors express receptors for this cytokine [9]. These data suggest that IL-5 signalling can, in some way, differ from signal transduction by the IL-3 or GM-CSF receptors.

3. Is there a role for βc in mediating signal specificity?

Dimerisation is a common theme in the activation of cell surface receptors [10] and cytokine receptors also depend on dimerisation for their activation. Studies using chimeric receptors consisting of the intracellular domains of the GM-CSF α and β chains fused to the c-*jun* or c-*fos* leucine zipper motifs have shown that both homodimers of βc intracellular domains and heterodimers of GM-CSFRα and βc can induce cytokine independent proliferation [11]. The stoichiometry of the endogenous active receptor complex is however unclear, although modelling studies suggest that the active receptor complex consists of two $\alpha\beta$ heterodimers [12].

Upon dimerisation, multiple tyrosine residues in the βc become rapidly phosphorylated [2,3](Fig. 2). Two membrane-proximal domains, including a conserved proline rich domain, were shown to be critical for

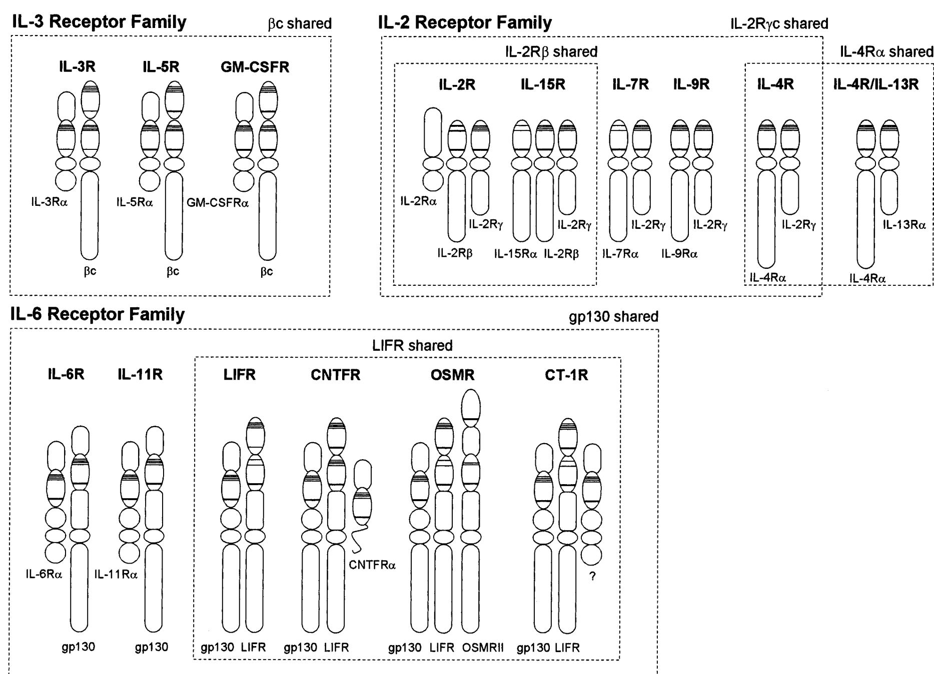

Fig. 1. Cytokine receptor class I subfamilies. Schematic representation of the molecular structure of class I cytokine receptors. The three major receptor families are depicted and these are subdivided according to the subunit composition of the receptors.

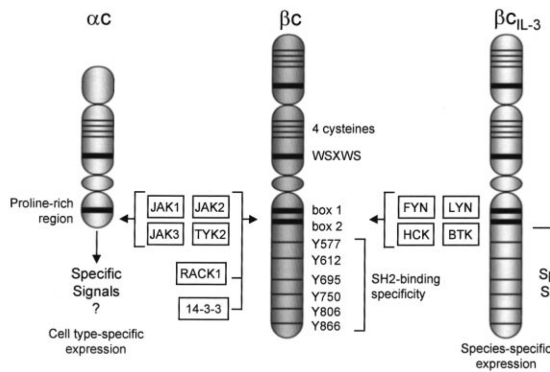

Fig. 2. Potential regulators of cytokine specificity in the IL-3 receptor family. The regulation of cytokine specific signal transduction can occur through a variety of mechanisms which are discussed in the text. The cell-type specific expression of α-chain subunits directly regulates the response of a cell to a specific cytokine. Furthermore, the intracellular domains of the different α-chains are likely candidates for mediating specific signals. A variety of signal transduction components that may interact with the α- and/or β-chains in a cytokine or cell-type dependent manner are shown (boxed). Finally the various βc phosphorylated tyrosine residues are depicted, each of which may bind specific SH2-domain containing proteins.

ligand-induced phosphorylation events [13]. This tyrosine phosporylation is thought to be mediated by members of the Janus kinase (JAK) family. Indeed, Jak2 has been shown to be constitutively associated with βc in a conserved region designated the Box 1 domain [14]. Receptor dimerisation will thus bring two Jak2 molecules into close proximity, which enables cross-phosphorylation and activation of the Jak2 proteins themselves, as well as phosphorylation of the dimerised receptor subunits. There are four members of the JAK kinase family; Jak1, Jak2, Jak3 and Tyk2 and they demonstrate some specificity in substrate phosphorylation [15]. Differential expression of JAK kinases could lead to cell type specific association of different members of the JAK family with βc, regulating the activation of specific downstream targets (see Fig. 2).

Phosphorylated tyrosine residues are target binding sites for proteins containing Src-homology 2 (SH2) domains [16]. There is a distinct binding specificity for SH2 domains to phosphotyrosine residues allowing specificity in the association of proteins with βc versus other cytokine receptor subunits. There are eight tyrosine residues present in the human βc, six of which are conserved between the human and mouse βc (see Fig. 2). Over the last few years much effort has been put into the identification of signalling pathways regulated by these (phosphorylated) tyrosine residues. For example, we have recently demonstrated that the activation of the PI-3K-PKB and the Ras-ERK pathway by IL-3/GM-CSF/IL-5 critically depends on the integrity of tyrosines Y577 and Y612 of βc [17]. Lopez and co-workers have recently demonstrated that βc also contains a phopshoserine motif that interacts with the adaptor protein 14-3-3ζ [18]. This serine residue is inducibly phosphorylated by GM-CSF in myeloid leukemic cells and may subserve specialised functions associated with IL-3/IL-5/GM-CSF receptors. This suggests that serine phosphorylation may also play a critical role in the regulation of receptor function.

Several other tyrosine kinases have been reported to associate with βc, including Lyn, Btk, Tec, Fyn, and Hck [19–22]. Although their role in βc signalling is largely unknown, the restricted expression of some of them might provide a mechanism by which specificity in signalling through βc is achieved. For example, expression of the tyrosine kinase Btk, is predominantly in B cells, and this kinase might therefore mediate a specific signal upon activation of βc in these cells. Indeed, Koike et al. demonstrated that X-linked immunodeficient mice (XID), which have a missense mutation in the Btk gene, display impaired IL-5 signalling in B-cells, whereas the eosinophilic development in these mice is normal [23].

We have recently described the identification of a novel adapter protein RACK1 that associates with the IL-3/IL-5/GM-CSF βc [24]. RACK1 has been reported to associate with active PKCβ in a region termed the C2 domain [25]. Indeed, PKCβ was found in complex with βc upon stimulation with either TPA or IL-5 [24]. Since there is no evidence that RACK1 associates with other cytokine receptor subunits, the association of RACK1 with βc might regulate signal transduction pathways specific for the IL-3/IL-5/GM-CSF family of receptors. Interestingly, while RACK1 is expressed in monocytes, T and B cells and eosinophils, we observed that RACK1 is absent from human neutrophils [24]. Thus, βc activation might lead to a cell-type specific activation of signalling pathways downstream of RACK1.

4. Fine-tuning specificity through cytokine receptor α-chains

Although the βc of the IL-3/IL-5/GM-CSF receptors initiates signal transduction events specific for this cytokine family, as previously discussed it will apparently not distinguish between IL-3, IL-5 and GM-CSF. The cytokine specific α-chains on the other hand are likely candidates for mediating specificity in signalling between cytokines of one family. Compared to the βc, the IL-3Rα, IL-5Rα and GM-CSFRα intracellular domains are rather short (around 50 amino acids). There is however, some experimental evidence that the intracellular domain of these receptors contributes to receptor signal transduction. Indeed IL-3Rα, IL-5Rα and GM-CSFRα have been shown to play a critical role in receptor signal transduction, since deletion of the cytoplasmic domains of the α-chains does not affect high

affinity ligand binding, but abrogates receptor signalling [26–31]. Deletion of the intracellular domain of IL-5Rα has been shown to abrogate the IL-5-mediated growth of a transfected BaF3 cell line [26]. Furthermore, ligand induced activation of JAK kinases and tyrosine phosphorylation of intracellular proteins as well as the activation of STAT5 was impaired by deletion of the intracellular domain [26,27]. The intracellular domains of IL-3Rα, IL-5Rα and GM-CSFRα contain a highly conserved membrane-proximal proline-rich region that has been referred to as the Box1 region [32]. A region downstream of this proline rich domain which is less conserved between the IL-5Rα, IL-3Rα and GM-CSFRα, has also been shown to be critical for IL-5R mediated signalling [30]. In a recent report, Jak2 was also shown to interact with the IL-5Rα Box1 region, and the ability of mutants of IL-5Rα to support IL-5R signalling correlated with the ability to associate with Jak2 [33]. Although, the association with the JAK kinases was uniquely demonstrated for the IL-5Rα, the Box1 regions of IL-3Rα and GM-CSFRα have also been shown to fulfil a similar critical function in the support of proliferation of cytokine dependent cell lines [28,29,31,34,35]. A role for the α-chain in the induction of PKC activation has been described for the GM-CSF and IL-3 receptors [29,36]. Furthermore, a single report demonstrates a direct role for GM-CSFRα in the regulation of hexose uptake in a tyrosine phosphorylation independent manner. This suggests a direct signalling role for the α-chain although the mechanisms underlying this observation remain unresolved [37].

In another study, IL-5 induced proliferation of TF-1 cells could be inhibited using a phosphatase inhibitor, whereas GM-CSF or IL-3 induced proliferation was unaffected [36]. Conversely, the GM-CSF or IL-3 induced proliferation could be inhibited utilising tyrosine kinase inhibitors, whereas these inhibitors enhanced IL-5 induced proliferation. Differences in tyrosine phosphorylation patterns induced by IL-3 or GM-CSF have also been reported by Evans et al., utilising a multipotential hematopoietic precursor cell line (FDCP-mix cells), stably transfected with either the human IL-3R$\alpha + \beta$c or human GM-CSFR$\alpha + \beta$c [38]. Furthermore, although both IL-3 and GM-CSF supported rescue form apoptosis of these cell lines, only IL-3 stimulation resulted in proliferation [38]. Analysis of the cellular morphology of cells cultured in the presence of either IL-3 or GM-CSF revealed that cells cultured on IL-3 had maintained their blast cell morphology, whereas cells cultured on GM-CSF had differentiated towards a mature phenotype [38]. Although differences in signal transduction through the receptors for IL-3, IL-5, GM-CSF thus clearly exist, the nature of these differences remains as yet undefined.

5. Transgenes and knock-outs of the IL-3, IL-5, GM-CSF receptor system

In the past few years murine knockout studies of virtually all components of the IL-3, IL-5, GM-CSF receptor system have been described. These have helped to understand the specific contributions of each of the receptor subunits in hematopoiesis. Null mutation of the common βc rendered bone marrow cells derived from these mice unresponsive to both IL-5 and GM-CSF [39,40]. However, responses to IL-3 were normal, demonstrating that in mouse, βc and $\beta_{\text{IL-3}}$ are redundant in terms of IL-3 signal transduction. βc(-/-) mice show a reduction in eosinophil numbers in both peripheral blood and bone marrow [39,40]. The reduced numbers of eosinophils observed in βc(-/-) mice are likely to be the result of defective IL-5 signalling, since this phenotype was also observed in IL-5(-/-) and IL-5Rα(-/-) mice [41,42]. Interestingly, although eosinophil numbers in these animals are low, these cells are not absent, suggesting a role in the expansion of these cells whereas βc signalling is probably redundant in mediating eosinophil differentiation. In addition, mice have been generated in which the gene for $\beta_{\text{IL-3}}$ was deleted [39], as well as double knock-out mice, lacking both βc and $\beta_{\text{IL-3}}$ [43]. Mice lacking $\beta_{\text{IL-3}}$ develop normally, and bone marrow cells derived from these mice responded normally to both IL-5, GM-CSF as well as IL-3, again indicating that βc and $\beta_{\text{IL-3}}$ are redundant. Mice lacking both βc and mouse $\beta_{\text{IL-3}}$, show no additional defects compared to the βc mutant mice [43].

Since βc null mutants provide little information about the specific contributions of each cytokine to hematopoiesis, the IL-5 and GM-CSF signals have been specifically disrupted by deletion of either the gene for the receptor α-chain or the gene encoding the cytokine itself. GM-CSF(-/-) mice show a normal steady-state hematopoiesis suggesting that GM-CSF is redundant in this process [44,45]. The only abnormalities were found in the lung, showing progressive accumulation of surfactant lipids and proteins in the alveoli. Extensive lymphoid hyperplasia was observed around the lung airways and veins similar to that observed in βc (-/-) mice [46]. This could be due to dysregulated macrophage activation since these mice show a selective upregulation of macrophage number and function [47–49]

Disruption of IL-5 signalling revealed a more specific role for this cytokine in hematopoiesis. Both mice deficient in IL-5 and mice deficient in IL-5Rα demonstrated decreased numbers of eosinophils, similar to the βc(-/-) mice [41,42]. Furthermore, helminth induced eosinophilia was absent from IL-5(-/-) and IL-5Rα(-/-) mice, indicating that IL-5 plays an critical role in the expansion of the eosinphil population. Both IL-5(-/-) and IL-5Rα(-/-) mice had markedly reduced numbers

of CD5^{+} B cells (B1 cells) and thymocytes, although thymocyte numbers returned to normal levels when the mice were 6 weeks of age. Interestingly, this effect has not been documented for βc(-/-) mice, where peritoneal cell populations were [42]. It is, however, unclear whether this difference is due to a lack of experimental data, or that it represents a true discrepancy between βc(-/-) mice and IL-5Rα(-/-) or IL-5(-/-) mice. The opposite effect was observed in transgenic mice, expressing high levels of IL-5 [50,51], which resulted in an increase in peripheral white blood cells, spleen cells and peritoneal cells. Especially an increase in eosinophils and B-1 cells was observed. The increase in B-1 cells was accompanied by an increase in serum IgM and IgA levels. Analysis of null mutant mice has clearly demonstrated that specificity in IL-3, IL-5 and GM-CSF signalling is critical for normal hematopoietic functioning (Table 1).

6. Conclusions

The development of novel molecular tools such as the yeast two-hybrid system have made the identification of specific proteins interacting with the IL-5, IL-3 or GM-CSF receptor α-chain a possibility. Furthermore, knock-in studies using mutants of receptor α-chains will provide additional information on the role of specific domains of these receptor subunits in the phenotype observed in the knock-out mice. Our current knowledge of the temporal and spatial expression of both the cytokines and their receptors, as well as the possible differences in expression levels and internalisation kinetics between the IL-3, IL-5 and GM-CSF receptors must be expanded. These might still be a critical mechanism by which specificity in IL-3, IL-5, GM-CSF receptor signalling is achieved through modulation of signal strength. With a greater understanding of the precise role of cytokine-specific receptor subunits the development of novel strategies for modulating cell function can be developed. Efficiency is realised when signal transduction pathways are inhibited at the point of receptor-mediated initiation preventing a signal from becoming amplified. Several methods have been developed that allow inhibition of the synthesis of specific proteins using anti-sense oligonucleotides or abrogation of specific protein-protein interactions using peptides mimicking the protein binding site. The inhibition of cytokine receptor α-chain mediated signal transduction pathways utilising for example peptides, derived from the α-chain intracellular domains might provide a novel way to specifically inhibit hematopoietic cell function. Specifically, the ability to inhibit cytokine-mediated proliferation and survival of subsets of hematopoietic cells can have important therapeutic implications for modulating a variety diseases.

Table 1
Phenotypes associated with gene disruptions of specific components of the IL-3/IL-5/GM-CSF receptor system[a]

	IL-5 (-/-)	IL-5Rα (-/-)	GM-CSF (-/-)	βc (-/-)	β_{IL-3} (-/-)	βc (-/-)+β_{IL-3} (-/-)
Apparently normal steady state hematopoiesis	✓	✓	✓	✓	✓	✓
Low eosinophil counts	✓	✓		✓		✓
No eosinophil induction upon infection or allergen challenge	✓	✓		✓		✓
Reduced mucosal IgA levels	x	✓				
Normal serum IgA and total IgG levels		✓				
Normal IgM, IgA and IgG responses upon antigen challenge or infection	✓					
Reduced serum IgM and IgG3 levels		✓				
Decreased CD5^{+} B cell levels	✓	✓				
Decreased thymocyte levels		✓				
Pulmonary proteinosis			✓	✓		✓
Impaired adhesion and reduced phagocytosis by alveolar macrophages			✓	✓		✓
Peritoneal macrophages normal				✓		
No colony formation from bone marrow cells in response to IL-5 or GM-CSF				✓		✓
IL-3 response normal		✓		✓	✓	
Increased numbers of neutrophils in BAL fluid				✓		
Lymphoid hyperplasia around aiways and pulmonary veins			✓			

[a] Ticked boxes indicate the appearance of the indicated phenotype. A cross indicates the absence of the indicated phenotype. Open boxes indicate that the phenotype has not been reported, nor has the absence of this phenotype.

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