

● Introduction

Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interleukin-3 (IL-3) and IL-5 are structurally and biologically related cytokines which are important players in both hematopoiesis and inflammation. The receptors for GM-CSF, IL-3 and IL-5 are also related as members of the Type I Cytokine Receptor superfamily as defined by signature motifs in their extracellular domains and by common functional paradigms (1). The cell surface receptors for GM-CSF, IL-3 and IL-5 are heteromeric complexes com-

structurally and biologically related receptor complexes reveals both similarities and differences which hint at important roles in the control of the biological activity of their cognate ligands.

ogenous leukemia elevated levels of sGMR α predict for a specific clinical phenotype which includes older age, high white blood cell count (including myeloid blasts), an increased risk of unexplained lung infiltrates and hypoxia and lower rates of microbologically defined infections (10). A GM-CSF binding moiety has also been identified in the bronchoalveolar lavage (BAL) fluid of patients with Pulmonary Alveolar Proteinosis (11), a rare respiratory failure state in which functional GM-CSF deficiency is known to play a causative role (12-14). Moreover we have found sGMR α in synovial fluid from patients with a variety of arthritic conditions with the highest levels correlating with a diagnosis of Rheumatoid Arthritis (Poubelle and Brown, unpublished).

Until recently, human soluble IL-5R α (sIL-5R α) protein levels in

Soluble Receptor Variants in the GM-CSF, IL-3, IL-5 Receptor Family

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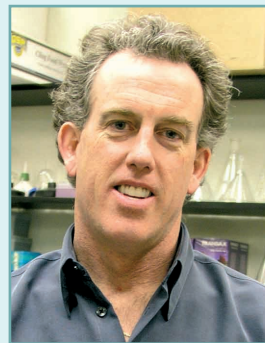
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posed of low affinity, cytokine-specific " α -subunits" (GMR α , IL-3R α , IL-5R α) and a shared signal-transducing " β subunit": β -common (β c). β c has no intrinsic cytokine binding function but is recruited by the α -subunits upon ligand binding, which shifts the complex to a higher affinity state. Sharing of β c largely explains the extensive overlap in the biological capabilities of GM-CSF, IL-3 and IL-5 and has served to define these three receptors as a functional subgroup amongst the larger Type I Cytokine Receptor family.

Many Type I Cytokine Receptors have soluble isoforms that can act as agonists and/or antagonists of the biological activity of the cytokines to which they bind. Over the years, a number of excellent reviews of general soluble cytokine receptor biology as well as the role of soluble cytokine receptors in human disease have been written (the reader is directed to: (2-9)). This review aims to more narrowly summarize data on the existence, origins and functions of soluble isoforms of the human receptors for GM-CSF, IL-3 and IL-5. Available information regarding soluble isoforms of these three

● Evidence of Existence In vivo

A soluble variant of GMR α (sGMR α) is a normal constituent of human



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serum with a normal range of 10-85 pM. sGMR α levels are associated with several disease states. Soluble GMR α serum levels are dysregulated in a statistically significant proportion of adults with hematological malignancies. Serum levels are elevated in over 40% of patients with myelodysplastic syndrome and patients with acute myelogenous leukemias of the M4 and M5 FAB subtypes. Conversely 20% of patients with acute lymphoblastic leukemia have levels below normal or even undetectable. In acute myel-

biological fluids had not been reported. However, a sIL-5R α ELISA has now been utilized to measure sIL-5R α levels in concentrated BAL fluids (15). This study demonstrated very low basal levels of sIL-5R α in BAL fluids from atopic individuals, and further noted that the concentration of sIL-5R α was elevated following antigen challenge. It is anticipated that this new ELISA will soon be used to determine whether sIL-5R α , like sGMR α , is found circulating at significant baseline levels in normal human serum.

● Mechanisms of Production and Cellular Sources.

The alternatively spliced soluble GMR α (alternatively spliced sGMR α)

Following the initial cloning of the cDNA encoding the membrane-spanning human GMR α in 1989 (16), a putative sGMR α variant was cloned which accounted for approximately 20% of the total GMR α mRNA in normal cells (17,18). Determination of the gene structure of human GMR α confirmed that this alternatively spliced sGMR α message (as-sGMR α) results from the exclusion of exon 11 of the GMR α gene, which encodes solely the transmembrane portion of this receptor subunit (19) (see Figure 1).

macrophages, and rheumatoid synovial fibroblasts (20). In fact, to date it appears as though all cells examined which bear cell surface GM-CSF receptors also contain the mRNA encoding alternatively spliced sGMR α . Importantly, however, there is evidence to suggest that transcription of the full-length and soluble GMR α mRNAs can be differentially regulated during myeloid differentiation towards the neutrophil lineage (22), which suggests a functional role for sGMR α in GM-CSF receptor signaling.

Our lab has recently developed an sGMR α splicing-specific ELISA and used it to confirm that translation of the as-sGMR α transcript and secretion of as-sGMR α protein does indeed occur in human monocytes (23).

(including sGMR α) requires the alternative splicing—or “skipping”- of the exon encoding the transmembrane region of the full-length receptor, it is a constitutive splicing event which leads to production of sIL-5R α , and an alternative splicing event (whereby the C-terminal exon of sIL-5R α , which contains a stop codon, is skipped) which is required for generation of transmembrane IL-5R α (24,26) (see Figure 1). It has been demonstrated that there are significantly higher levels of sIL-5R α mRNA in the bronchial mucosa of asthmatic individuals versus non-asthmatic control subjects (27), supporting the importance of this form of the soluble IL-5R α .

Ectodomain cleavage of the membrane-bound receptor subunits

The soluble GM-CSF and IL-5 receptors have traditionally been thought to be generated exclusively through differential splicing. However, recent studies have revealed the existence of further sGMR α and sIL-5R α isoforms: variant proteins which appear to be generated through the metalloprotease-mediated ectodomain cleavage of the membrane-bound receptor α -subunits.

Our own lab has recently demonstrated that monocytes treated with a broad spectrum metalloprotease inhibitor (BB94) secreted less total sGMR α protein than did untreated monocytes, but that BB94 itself had no effect on the secretion of as-sGMR α protein. These results suggest that a second soluble GM-CSF receptor species arises through metalloprotease-mediated proteolysis of the extracellular domain of GMR α , a hypothesis corroborated by recombinant GMR α models (23).

In the case of sIL-5R α , it was noted that levels of IL-5R α on the surface of eosinophils were decreased following airway antigen challenge or direct stimulation with IL-5. This was accompanied by an increase in sIL-5R α levels released into the surrounding extracellular fluids (15,28). Since these observations were suggestive of IL-5R α ectodomain shedding, purified eosinophils were then pretreated with a variety of metalloprotease inhibitors prior to stimulation with IL-5. This led to the partial inhibition of membrane IL-5R α loss and decreased accumulation of sIL-5R α in the

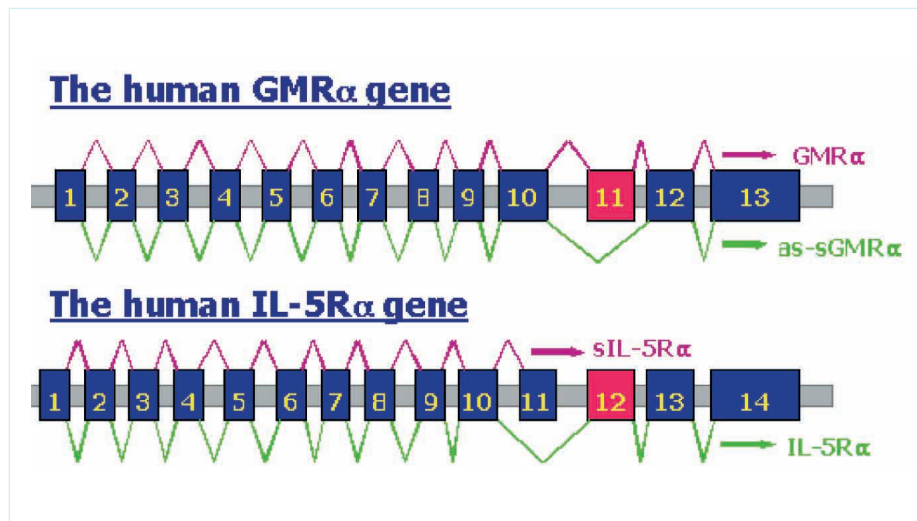


Figure 1: Generation of transmembrane-spanning and soluble isoforms of the GM-CSF and IL-5 receptor alpha subunits by differential splicing. The genomic structures of the GMR α (19) and IL-5R α (26) genes are shown. Exons are represented by blue boxes and are numbered. Exons containing the transmembrane domains are indicated in red. The generation of the indicated receptor isoform by constitutive splicing is represented in pink, whereas the generation of a specific receptor isoform by alternative splicing is represented in green. In the case of GMR α , it is an alternative splicing event which leads to formation of a soluble receptor species, whereas for IL-5R α , sIL-5R α is generated by constitutive splicing. (Please note that exon and intron sizes are not drawn to scale).

The alternative splicing event results in a shift in reading frame, such that the extracellular domain of GMR α is retained, while the transmembrane and cytoplasmic domains are replaced by a unique 16 amino acid C-terminal tail (Leu Gly Tyr Ser Gly Cys Ser Arg Gln Phe His Arg Ser Lys Thr Asn) (16-18).

The as-sGMR α transcript is widely distributed, its existence being demonstrated in a variety of cell types, including myeloid leukemic cell lines (18,20), human placental tissue (21), bone marrow, monocytes/

The naturally occurring soluble IL-5R α (sIL-5R α) transcript

In contrast to the alternatively spliced sGMR α transcript, which is a minor species as compared with the transmembrane form of GMR α , the primary human IL-5R α transcript encodes for a soluble IL-5R α . In human eosinophils, as much as 95% of the total IL-5R α mRNA represents the soluble isoform (24,25). This finding may be explained in part by looking at the IL-5R α gene structure. Whereas generation of most soluble receptors

extracellular space consistent with generation of sIL-5R α by a process of ectodomain shedding (15).

● Cellular sources

To date there is immunological, biochemical and functional evidence that human sGMR α protein can be expressed by several myeloid leukemic cell lines (HL60, THP-1, U937), neutrophils, and monocytes (23,29), and immunological evidence of production by some primary leukemic blasts (10). In monocytes production can be upregulated by a variety of inflammatory stimuli including GM-CSF itself (23). The cellular sources of sIL-5R α have not been extensively catalogued with available evidence so far restricted to eosinophils. Production of sIL-5R α by eosinophils is also upregulated by its cognate ligand (28). For both sGMR α and sIL-5R α it is unclear what the balance of production is between the alternatively spliced and proteolytically cleaved isoforms of the soluble receptors. The recent development of antibodies which specifically recognize the alternatively spliced sGMR α should allow clarification of this. Preliminary results suggest that total sGMR α levels in human serum do represent a mixture of both spliced and shed isoforms of soluble GMR α (Pelley, unpublished).

● Influence of the Soluble Receptors on Cytokine Activity

Soluble GMR α binds to GM-CSF in solution phase thus preventing GM-CSF interaction with the cell surface GMR α / β c complex (21). As a consequence sGMR α has been shown to inhibit the proliferation of GM-CSF-dependent cell lines (18,20) and bone marrow colony formation (21).

Soluble IL-5R α also demonstrates antagonistic properties by preventing the binding of IL-5 to the IL-5R α / β c complex on the surface of human eosinophils (30). The soluble IL-5R α thus inhibits IL-5-mediated signal transduction, eosinophil differentiation, proliferation, survival, and priming of inflammation (25,30,31).

Given that the soluble receptors must sequester their respective ligands away from the high affinity cell-surface receptors it is instructive to compare the binding affinities of the soluble and cell-surface receptor complexes. Table 1 illustrates that sIL-

5R α binds to IL-5 with an affinity that far more closely rivals that of the „high affinity“ IL-5R α / β c complex than does sGMR α as compared with GMR α / β c. This would predict that, on a molar basis and within their respective systems, soluble IL-5R α is a more efficient antagonist than soluble GMR α .

On the other hand there is evidence that cell surface GMR α alone mediates some cellular signaling in the absence of β c. For instance, GMR α on its own may signal for increased glucose uptake via the PI 3-kinase pathway (32,33). GMR α is also present in the absence of β c on the surface of placental (16) and embryonic tissues (34) and appears to play a role in both placental (35) and embryonic growth and survival (34). In these situations soluble GMR α would provide a far more potent modulating effect.

Shedding of GMR α and IL-5R α from the cell surface to create soluble receptors (15,23) provides another means of cytokine antagonism by the removal of functional receptor complexes from the cell surface. However this antagonism would be dependent on the efficiency of removal of the cell-surface subunits. Importantly, treatment of eosinophils with IL-5 leads to an almost complete release of IL-5R α from the cell surface (15). However, in our hands, induction of GMR α shedding by monocytes does not lead to an appreciable diminution in the cell surface levels of GMR α as measured by flow cytometry (Prevost and Pelley, unpublished) suggesting that only part of the population of GMR α is subject to proteolytic shedding.

Although a substantial amount of *in vitro* evidence supports a role for sGMR α and sIL-5R α as antagonists of their respective cytokines, it is more difficult to predict what role these soluble receptors will play *in-vivo*. This difficulty is illustrated by the soluble TNF receptor (sTNFR), which acts as a strict antagonist of TNF function *in vitro*, but which was demonstrated in mouse models to act as both an antagonist and as a carrier protein which increased TNF levels in serum and enhanced TNF activity overall (36). The balance between antagonism and carrier properties may lie in the ratio of ligand to soluble receptor (37), and this balance may have considerable clinical significance (38).

In the IL-6R family of receptors soluble α -subunits act as agonists by delivering their respective ligands to isolated signal transducing subunits on the cell surface to form signaling-competent complexes (39). Such “trans” signaling does not occur between exogenously added sGMR α (40) or sIL-5R α (25) and the cell-surface β c subunit. However, in a recombinant cell system, as-sGMR α is retained on the cell surface through a disulfide bond between the 16 amino acid tail of as-sGMR α and the β c subunit, provided that as-sGMR α and β c are expressed in the same cell (40,41). The as-sGMR α / β c complex binds GM-CSF with high affinity (41), but does not seem capable of transmitting a signal (Zhu, unpublished) and it thus may serve as a decoy receptor. To date this complex has not been identified *in vivo* so its physiological relevance is unclear.

Finally, soluble forms of gp130 and γ c, the common signal-transducing subunits of the IL-6 and IL-2 receptor families respectively have been described (42-44) and are able to act as antagonists of cytokine signaling by combining in solution phase with both their cytokine ligand and the soluble receptor α -subunit (42,44-46). In this regard an alternatively spliced β c variant mRNA has been reported which is predicted to encode only a membrane-distal extracellular portion of β c (47) but there has been no report of a protein product of this transcript. There also exists the unexplored possibility that a soluble form of β c, like sGMR α and sIL-5R α , could be produced through ectodomain shedding.

● Where is the Soluble IL-3 Receptor?

In contrast to the IL-5 and GM-CSF receptors, there are no reports to date of a soluble variant of IL-3R α in either human or mouse. Examination of the genomic sequence of IL-3R α (48) indicates that a soluble alternatively spliced IL-3R α species could be generated in a manner analogous to sGMR α . Based on a lack of a minimal consensus shedding sequence (49), it is impossible to predict whether the IL-3R α subunit might be proteolytically cleaved to yield a shed sIL-3R α variant.

The cell surface IL-3R α possesses a relative affinity for ligand

Receptor for	Kd soluble receptor	Refs	Kd alpha alone	Refs	Kd alpha/beta	Refs
GM-CSF	2-10 nM	(21)	2-8 nM	(16)	20-100 pM	(50-53)
IL-3	ND	-	120 nM	(48)	70-140 pM	(48, 54)
IL-5	180-290 pM	(55)	860 pM	(24)	240-470 pM	(24, 56)
	800 pM	(30)	440 pM	(30)	600-700 pM	(30)

Table 1: Affinities of the soluble receptors, low affinity receptors, and high affinity receptors of the GM-CSF/IL-3/IL-5 receptor subfamily for their respective ligands.

which is 1000-fold less than that of the high-affinity IL-3R α / β c (see Table 1). Hence a soluble form of IL-3R α would be a very poor antagonist indeed; this fact may play a role in the selection pressures influencing the stable evolution of such a molecular species.

● Summary

Much remains to be understood about the biology of soluble variants of the receptors for GM-CSF and IL-5. Even the lack of evidence of a soluble IL-3 receptor provides the opportunity to gain insight into the complexities of cytokine signal modulation offered by these molecules. Their ability to alter

cytokine activity also offers the possibility to utilize these soluble receptors as therapeutic reagents - a challenge for future study.

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