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# The Structure of the GM-CSF Receptor Complex Reveals a Distinct Mode of Cytokine Receptor Activation

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## SUMMARY

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a pleiotropic cytokine that controls the production and function of blood cells, is deregulated in clinical conditions such as rheumatoid arthritis and leukemia, yet offers therapeutic value for other diseases. Its receptors are heterodimers consisting of a ligand-specific α subunit and a βc subunit that is shared with the interleukin (IL)-3 and IL-5 receptors. How signaling is initiated remains an enigma. We report here the crystal structure of the human GM-CSF/GM-CSF receptor ternary complex and its assembly into an unexpected dodecamer or higher-order complex. Importantly, mutagenesis of the GM-CSF receptor at the dodecamer interface and functional studies reveal that dodecamer formation is required for receptor activation and signaling. This unusual form of receptor assembly likely applies also to IL-3 and IL-5 receptors, providing a structural basis for understanding their mechanism of activation and for the development of therapeutics.

## INTRODUCTION

A common feature of cytokine receptor activation is ligand-induced receptor aggregation involving the homo or heterodimerization of two or more receptor components and their assembly into a fully functional signaling complex. Structural data for heterodimeric receptor families that utilize a common binding and signaling subunit such as the IL-2 and IL-6 receptor systems have provided unique insights into their functional activation (Boulanger et al., 2003; Wang et al., 2005; Stauber et al., 2006). The GM-CSF, IL-3, and IL-5 family of receptors remains the last major group of class I hematopoietic receptor systems to be structurally and functionally elucidated.

The GM-CSF, IL-3, and IL-5 family of cytokines regulates the survival, proliferation, differentiation, and functional activation of hematopoietic cells (Guthridge et al., 1998) with GM-CSF also controlling dendritic cell (Mellman and Steinman, 2001) and T cell function (Barouch et al., 2002), thus linking innate and acquired immunity. While on the one hand GM-CSF offers therapeutic promise to bolster antitumor immunity (Sun et al., 2002; Fleetwood et al., 2005) and innate immunity for the treatment of Crohn's disease (Korzenik et al., 2005), on the other hand abnormalities in GM-CSF production or receptor function have been implicated in multiple pathologies such as rheumatoid arthritis (Cook et al., 2001), juvenile myelomonocytic leukemia (Birnbaum et al., 2000), chronic myelomonocytic leukemia (Ramshaw et al., 2002), and alveolar proteinosis (Dirksen et al., 1998). Furthermore, the GM-CSF receptor may also be important in the pathogenesis of chronic myeloid leukemia and myeloproliferative diseases by propagating survival and proliferation signals promoted by the abnormal expression of Bcr-Abl and JAK2 mutations, respectively (Wilson-Rawls et al., 1996; James et al., 2005). The receptors for GM-CSF, IL-3, and IL-5 are expressed at very low levels (100–1000 per cell) on the surface of hematopoietic cells and comprise a cytokine-specific α subunit and the βc subunit that is common to all three receptors (Guthridge et al., 1998). Each α subunit binds cytokine with low affinity ($K_D$ = 0.2–100 nM) but the presence of βc converts this to high affinity ($K_D$ = 100 pM) causing dimerization of both subunits and receptor activation (Stomski et al., 1996). Structure-function studies of GM-CSF, IL-3, and IL-5 and their receptors have noted regions of importance for ligand binding and biological activity; however the composition, assembly, and underlying mechanisms of receptor activation have remained elusive.

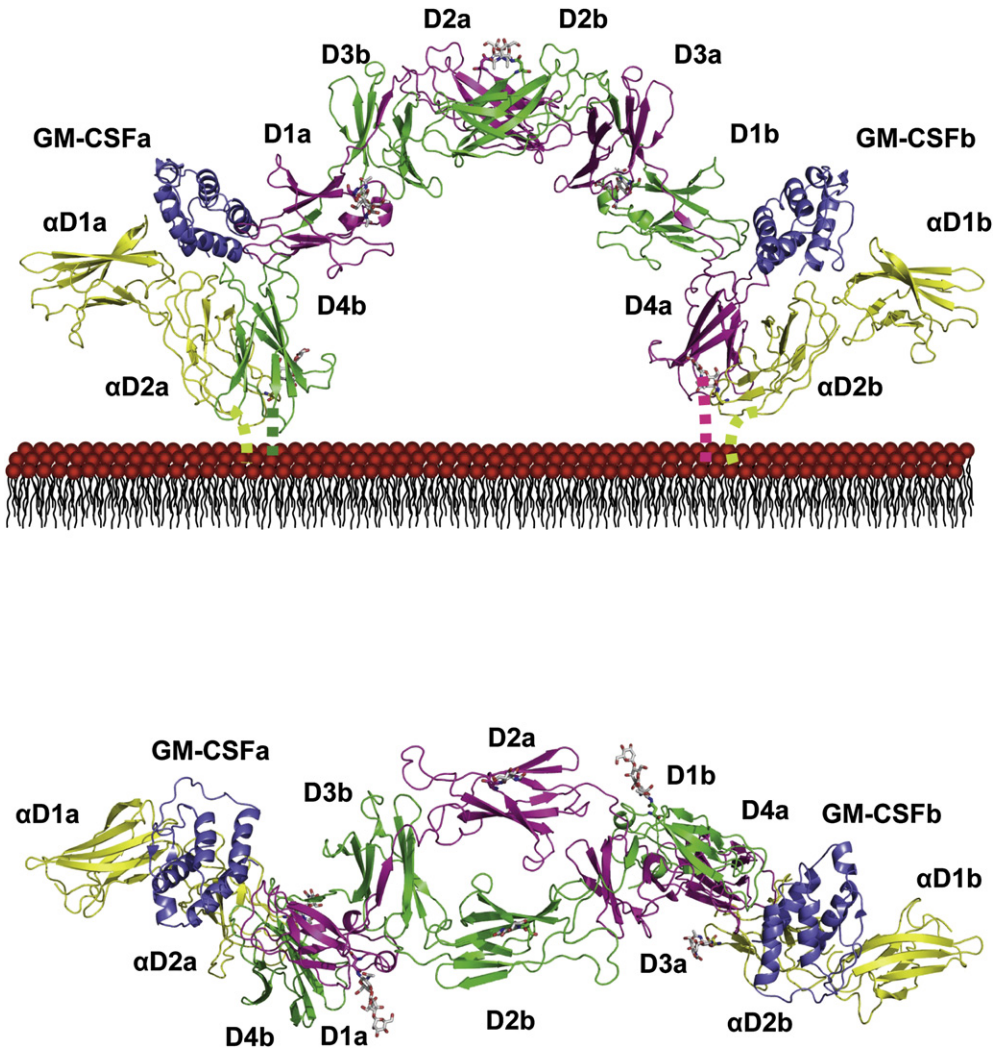


**Figure 1. Structure of the GM-CSF Receptor Ternary Complex**

GM-CSF is highlighted in blue and GMRα in yellow. One monomer of βc is shown in magenta (chain a) and the other in green (chain b). Labels correspond to domain names. Orthogonal views show how the complex would sit on the membrane surface. The bottom panel shows the view of the receptor when looking toward the membrane and the top panel shows a side-on view with the molecule sitting on a membrane surface. Observed N-linked carbohydrates are shown as sticks. Disordered peptides that connect the C termini of each chain to the membrane are shown as dashed lines. This and the following figures were prepared with PyMOL (DeLano, 2002).

Although GM-CSF receptor activation follows general rules that invoke receptor dimerization and tyrosine transphosphorylation of the cytoplasmic domains (Schlessinger, 2000), it is not clear how this is achieved. The GM-CSF receptor does not have intrinsic tyrosine kinase activity but associates with the tyrosine kinase JAK2, which is required for βc transphosphorylation and the initiation of signaling and biological activity. The cytoplasmic domains of both GMRα and βc are essential for receptor activation (Sakamaki et al., 1992; Muto et al., 1995), but it appears that mainly βc associates with JAK2 (Brizzi et al., 1994; Quelle et al., 1994; Lilly et al., 2001). Since the crystal structure of isolated βc revealed a dimer in which the membrane-proximal domains were 120 Å apart (Carr et al., 2001), a distance too great to allow transphosphorylation of βc by their associated JAK2 kinases, it has remained an enigma how activation of the GM-CSF, IL-3, and IL-5 family of receptors could be achieved. We show here that the crystal structure of the GM-CSF ternary complex assembles into an unexpected dodecamer arrangement. Functional analyses show that this dodecamer or higher-order complex brings two βc dimers into close proximity and provides for the functional dimerization and activation of the GM-CSF receptor.

## RESULTS

### The Structure of the GM-CSF Receptor Complex Reveals a Hexameric Assembly

We have determined the structure of the GM-CSF ternary complex revealing a 2:2:2 hexamer consisting of two βc chains, two GMRα chains, and two GM-CSF molecules (Figure 1). This differs from the 2:1:1 stoichiometry (2 βc: 1 GMRα: 1 GM-CSF)