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Original Paper

Molecular Aspects of the GM-CSF Receptor: An Example of the Cell Signalling Mechanisms Used by Type 1 Cytokine Receptors

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INTRODUCTION

THE TYPE 1 cytokine receptor super family is now very large. Amongst the best characterised of the family members are the prolactin and growth hormone receptors reviewed in [1]. Several of the properties of cell signalling intermediates, to be described for the granulocyte macrophage-colony stimulating factor (GM-CSF) receptor, were originally described from work on the regulation of milk protein synthesis by prolactin.

RECEPTOR STRUCTURE

Studies on the physiological events induced by GM-CSF showed that, in cells with the appropriate receptors such as eosinophils and basophils, GM-CSF induces the same responses as IL-3 and IL-5 [2, 3]. From this observation, it was quickly realised that the receptors for all three cytokines share a common sub-unit. It was established [4, 5] that all three receptors are heterodimers in which there is a common β-sub-unit. Correspondingly, receptor specifity arises from the interaction of ligand with the α-sub-unit. All three ligands, GM-CSF, IL-3 and IL-5 have four α-helical regions [6]. The region nearest the N-terminal end of the molecule interacts specifically with the common β-sub-unit of the receptor [7], whereas the α-helical domain nearest the C-terminal end of the ligand confers the receptor specificity by binding only to the α-sub-unit specific for its own receptor. The existence of a common sub-unit amongst a family of plasma membrane receptors has long been recognised in the case of the gonadotrophins in which receptors for luteinising hormone, follicle stimulating hormone, β-hCG and also thyroid stimulating hormone all have a common β-sub-unit which is involved in activating adenyl cyclase through a G-protein. It is not, therefore, surprising to find that, in the case of the receptors for GM-CSF, IL-3 and IL-5, it is the β-sub-unit which initiates the cell signalling process.

CELL SIGNALLING MECHANISMS

Once the ligand is bound by the external domain of the receptor, the internal domain of the β-sub-unit is able to recruit Jak-2 (a member of the Janus family of tyrosine kinases; Figure 1). The internal domain of the β-sub-unit of the receptor contains two distinct motifs known as box-1 and box-2. Box-1 is located immediately adjacent to the internal face of the plasma membrane, whereas box-2 is located nearer to the C-terminal (internal) end of the domain. Jak-2 is recruited to the region of box-1, after which it then phosphorylates certain tyrosine molecules within the cytoplasmic domain of the β-sub-unit [8, 9]. Subsequent to this essential step, other soluble, cytoplasmic proteins are tyrosine phosphorylated as the cell signalling process gets underway.

One of the pathways activated is the *ras*/*raf* pathway leading to activation of MAP kinase followed by activation of c-*fos*, though there is still some argument about both the main pathway of *ras* activation and the relationship between *ras* activation and subsequent *fos* activation. From the point of view of understanding GM-CSF function, it is perhaps most important to look at nuclear events and so this part of the review will concentrate on the components of the receptor that are involved in the activation of c-*fos*. Nuclear responses are presumably the ones which foreshadow changes in both growth and differentiation of target cells.

In order to determine which of the tyrosines in the β-sub-unit was (the most) important in activating c-*fos*, Itoh and colleagues [10] carried out a series of elegant experiments using β-sub-units from which increasing lengths had been deleted. The modified β-sub-units were transfected, along with active α-sub-unit, into cells containing the c-*fos* promoter constructed onto a luciferase reporter gene. Deletions up to amino acid 589 (Δ-589) did not reduce c-*fos* activation by added ligand, whereas deletion at amino acid 544 caused complete loss of activity. There is only one tyrosine within the sequence 544–589 and so this (amino acid 577) is now considered to be the prime tyrosine whose phosphorylation must occur before the *ras*/*raf* pathway can be activated. A construct of Δ-589, in which tyrosine-577 has been substituted with phenylalanine, has no activity in the c-*fos* assay, confirming this observation. However, if tyrosine-577 is replaced by phenylalanine in the intact β-sub-unit, then almost full activity is retained, implying that other tyrosines can take on the role of 577 in the intact protein.

It can be argued that the c-*fos* pathway is less important than that which induces the activation of c-*myc* since the

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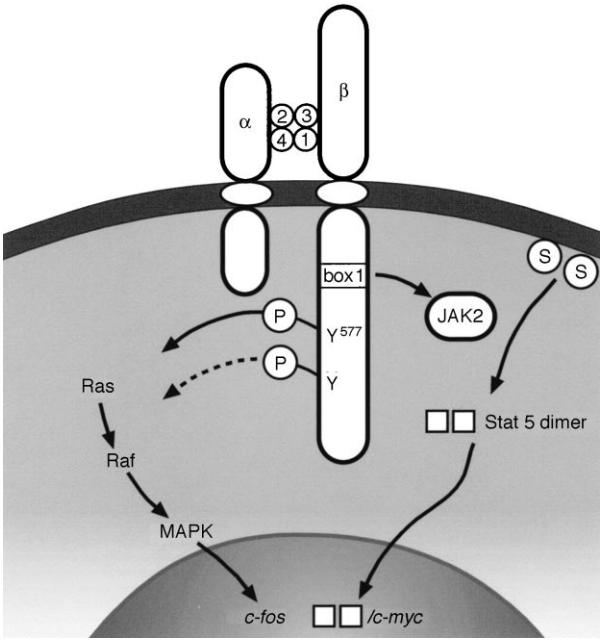

Figure 1. Summary of the overall action of the GM-CSF receptor in regulating nuclear events. Binding of ligand (represented by the four α-helices 1, 2, 3, 4) involves selective interaction with the α-sub-unit of the receptor to induce ligand-specific responses. The β-sub-unit, common to the whole family of receptors, is then activated to initiate intracellular signalling. This involves recruitment of the JAK2 kinase to a region (box 1) close to the membrane. As a result, several tyrosines, of which tyrosine-577 is the most important, become phosphorylated. This leads to the subsequent activation of the *ras*/*raf*/MAP kinase, leading to various cytoplasmic events, as well as up-regulating nuclear proteins, such as c-*fos*. JAK2 also recruits STAT 5 molecules and the activated STAT 5 dimer then translocates to the nucleus where it directly activates the c-*myc* gene, amongst others. The JAK/STAT and *ras*/*raf* pathways are thought to interact at several levels.

latter is critical to the induction of cell division. Activation of c-*myc* by the GM-CSF receptor occurs independently of the *ras*/*raf* pathway and, instead, is associated with the activation and dimerisation of stat-5 by Jak-2 (Figure 1) [11]. The stat-5 (stat is short for signal transducer and activator of transcription) dimer enters the nucleus and binds directly to specific nucleotide sequences in the promoter regions of target genes within the DNA, inducing transcription of genes such as oncostatin M [12], in addition to c-*myc*. Our current knowledge does not really permit a useful argument about the relative importance of these signalling pathways because there is considerable interconnection between the Jak-Stat and MAP kinase pathways [13].

Given the importance of the role of GM-CSF in the differentiation and function of granulocytes, macrophages and eosinophils, it was of interest to observe the fate of GM-CSF receptor knockout mice. Surprisingly, these mice develop to adult state with no apparent disadvantage. One possibility is that the main role of GM-CSF is in inducing reaction to infection, rather than in normal growth. However, the GM-CSF receptor null mice coped just as well as the controls with both parasitic and bacterial infection [12]. This observation implies that the redundant nature of the signalling pathways is such that gradually maturing mice can cope without any GM-CSF-induced signalling. Such an observation does not, of course, imply that a fully differentiated adult could necessarily cope with the sudden loss of GM-CSF receptor function.

CONCLUSIONS

Table 1. Summary

- The GM-CSF receptor, like all class 1 cytokine receptors, is a heterodimer.
- Both sub-units traverse the plasma membrane.
- The β-sub-unit is common to GM-CSF, IL-3 and IL-5 receptors.
- Activation of the c-*fos* pathway occurs through *ras*/*raf* and Map kinase and requires prior phosphorylation of tyrosine 577 by Jak-2.
- Activation of the c-*myc* pathway (and related pathways) requires Jak-2 activation of the stat-5 pathway.
- The signalling pathways involved are often closely interconnected.

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