RESEARCH HIGHLIGHT

Signal transduction of interleukin-11 and interleukin-6 α-receptors

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The cytokines Interleukin (IL)-11 and IL-6 are important mediators that regulate differentiation and proliferation of immune cells. Both cytokines bind to unique non-signaling α-receptors (IL-11R and IL-6R, respectively), and the resulting cytokine/cytokine receptor complexes recruit a homodimer of the signal-transducing β-receptor glycoprotein (gp)130. Gp130 is expressed ubiquitously, whereas both α-receptors show a cell- and tissue-specific expression pattern, thus determining cellular responsiveness towards IL-6 and/or IL-11. Formation of the signaling complexes activates intracellular signaling cascades, most prominently the Janus kinase (Jak)/Signal Transducer and Activator of Transcription (STAT) pathway. In a recent paper published in Biochimie, we analyzed the signaling capacity of eight chimeric receptors consisting of different domains of IL-11R and IL-6R. Our results showed that the intracellular region, the transmembrane region or the stalk region can be swapped between the two receptors, as they are not essential to discriminate between the two cytokines. Selectivity of the two receptors is exclusively warranted by the cytokine binding module (CBM), which resides within the domains D1 to D3. These results underline a modular organization of IL-11R and IL-6R and a comparable signal transduction of both cytokines.

Keywords: Interleukin-11; Interleukin-6; IL-6R; IL-11R; gp130; Jak/STAT

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The cytokines Interleukin (IL)-11 and IL-6 fulfill pleiotropic activities in health and disease and control several physiological conditions [1-3]. They exhibit a four-helical-bundle fold, which is arranged in an up-up-down-down topology (Figure 1A). In healthy individuals, both proteins are only detected in small amounts of 1-10 pg/ml [4, 5]. However, under inflammatory conditions, IL-6 serum levels can rise dramatically and reach several µg/ml [5]. Specific inhibition of IL-6 has been shown to be beneficial in a plethora of inflammatory diseases, and the neutralizing antibody tocilizumab which blocks binding of IL-6 to its receptor is approved for the treatment of Castleman's disease, systemic juvenile idiopathic arthritis, polyarticular juvenile idiopathic arthritis and rheumatoid arthritis [6-9]. Recent evidence suggests that also the specific blockade of IL-11 signaling might be beneficial, e.g. for the treatment of gastric and colon cancer [10, 11].

To activate target cells, IL-11 and IL-6 bind initially to non-signaling α-receptors (IL-11R and IL-6R, respectively). The formation of IL-11/IL-11R and IL-6/IL-6R complexes activates intracellular downstream signaling pathways like

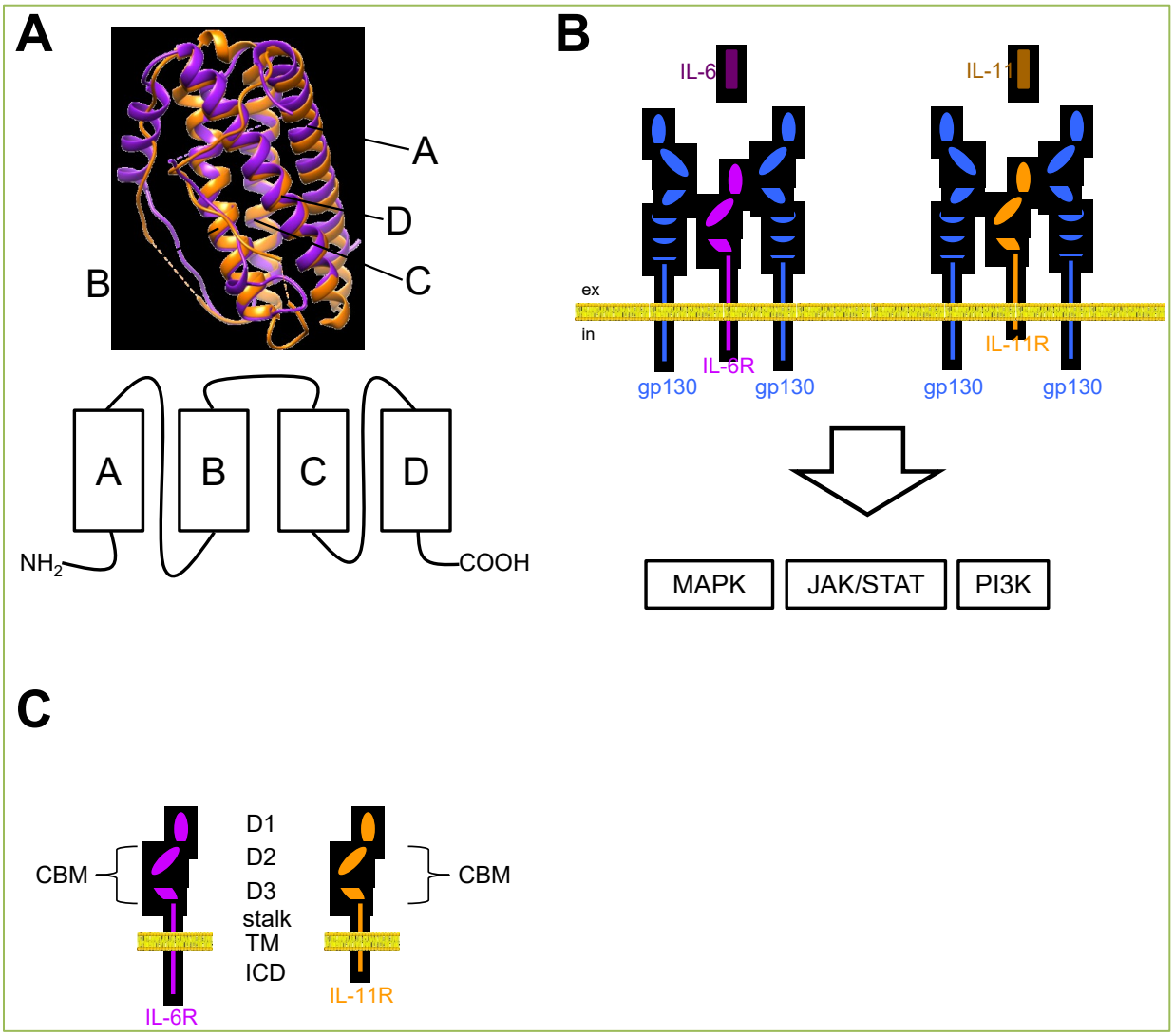

Figure 1. Signal transduction of IL-6 and IL-11. (A) Overlay of the crystal structures of IL-6 (pdb accession code 1alu [37], shown in purple) and IL-11 (pdb accession code 4mhl [38], shown in orange). The image was created using UCSF Chimera 1.10.1 [39]. The four helices A, B, C and D are indicated. The schematic drawing below shows the up-up-down-down topology of both cytokines. **(B)** Signal-transducing complexes of IL-6 (IL-6R/gp130/gp130) and IL-11 (IL-11R/gp130/gp130), which activate Mitogen-Activated Protein Kinase (MAPK), Janus kinase/Signal Transducer and Activator of Transcription (Jak/STAT) and Phosphoinositide 3-kinase (PI3K) signaling cascades. **(C)** Schematic drawing of IL-6R and IL-11R. The extracellular part consists of the three domains D1 to D3, and D2/D3 constitutes the cytokine-binding module (CBM). These are followed by a so-called stalk region, the transmembrane (TM) and the intracellular domain (ICD).

the Janus kinase/Signal Transducer and Activator of Transcription (Jak/STAT) cascade through engagement of a homodimer of the signal-transducing β-receptor glycoprotein 130 (gp130) [12] (Figure 1B). Although Jak1, Jak2 and Tyk2 are phosphorylated after activation of gp130 *in vitro*, experiments with cells derived from Jak1$^{-/-}$ mice have shown unequivocally that Jak1 is the dominant kinase activated by all members of the IL-6 cytokine family, the loss of which cannot be compensated by other kinases [13, 14]. Furthermore, the phosphatidyl-inositol-3-kinase (PI3K)-cascade and the mitogen activated protein kinase (MAPK)-cascade are activated [12] (Figure 1B). Gp130 is ubiquitously expressed, and specificity is thus achieved through cell- and tissue-specific expression of the α-receptors. The IL-11R can be found e.g. on cardiac myocytes and endothelial and epithelial cells of the colon, whereas the IL-6R is expressed e.g. on T cells, monocytes, neutrophils and megakaryocytes. Hepatocytes, B cells, macrophages and osteoclasts have been shown to express both receptors, which makes them a target of both cytokines (reviewed in [3]).

The IL-6R was cloned in 1988 [15] and the IL-11R in 1994 [16]. Both are type-I transmembrane proteins which consist of an extracellular part, a transmembrane region and an intracellular region (Figure 1C). Due to alternative mRNA splicing, a second membrane-bound IL-11R isoform exists in

humans that lacks the intracellular region [17], whereas alternative splicing of the IL-6R mRNA gives rise to a soluble form of the IL-6R [18]. Furthermore, a second IL-11R gene exists in mice, which is only expressed in lymph nodes, testis, and in the thymus [19]. The extracellular part of both receptors is composed of an Ig-like D1 domain, which is followed by the fibronectin-type-III domains D2 and D3 that comprise the cytokine-binding module (CBM), and a so-called stalk region (Figure 1C). We could show previously that an important function of the stalk region is to act as a brace in order to position the CBM of the IL-6R [20]. Although the conformation of gp130 is somewhat flexible [21-24], the CBM has to be kept in a certain distance from the plasma membrane [20]. A minimal length of approximately 83.6 Å of the stalk region, which corresponds to 22 amino acid residues, is required for efficient IL-6 signaling via the membrane-bound receptor, because IL-6R deletion variants containing a shorter stalk region were not biologically active, although they were correctly folded and transported to the cell surface [20]. Similar studies concerning the signaling properties of the IL-11R have not been conducted yet.

In our recent study [25], we analyzed the roles of the different parts of the IL-11R and IL-6R with respect to signal transduction. First, we examined whether a cross reactivity between IL-11 and IL-6 exists using genetically engineered Ba/F3 cell lines. Ba/F3 cells are murine pre-B cells that grow in strict dependence of IL-3, but can be rendered responsive to other cytokines via stable expression of the desired cytokine receptors. Using Ba/F3-gp130-IL-11R cells, we found that IL-6 could neither induce phosphorylation of STAT3 nor cell proliferation, indicating that IL-6 is not a high affinity ligand for the IL-11R. Vice versa, IL-11 could not activate Ba/F3-gp130-IL-6R cells. Taken together, we detected no cross-reactivity between IL-6 and IL-11.

Additionally, we created eight different chimeras of both receptors, where we left the three extracellular domains intact and exchanged either stalk, transmembrane, intracellular region, or all three parts between the two receptors and analyzed their responses to IL-6 and IL-11. We found that exchange of the stalk region between IL-11R and IL-6R did not alter their response towards the cytokines. Swapping the transmembrane or the intracellular part also did not affect signaling. Consequently, exchanging all three regions and keeping only D1-D3 intact still resulted in normal biological activity of the IL-11R or IL-6R, respectively. Notably, the origin of D1-D3 determined by which cytokine the chimeric receptors could be activated, while the other regions showed no influence. Thus, our results show a similar modular organization of IL-11R and IL-6R, which allows transfer of different regions between these receptors without interfering with their signaling capacity.

The intracellular region of the IL-6R contains 82 amino acid residues, the intracellular region of the IL-11R is remarkably shorter and contains only 32 amino acid residues. These regions do not participate in the signal transduction of both cytokines, because IL-11R and IL-6R mutants without their intracellular regions were fully biologically active [26, 27]. This finding is further corroborated by the fact that soluble forms of the IL-6R (sIL-6R) exist which are able to bind IL-6 with similar affinity as the membrane-bound IL-6R [18]. The resulting IL-6/sIL-6R complexes are able to activate cells via gp130 homodimerization irrespective of the presence of membrane-bound IL-6R, a fact that dramatically expands the number of cells which can be activated by IL-6 via so-called trans-signaling [28]. The sIL-6R originates to a minor extent from alternative mRNA splicing (~10 %), whereas the majority is believed to originate from proteolytic cleavage of the membrane-bound IL-6R (~ 90%) [18]. Among several proteases that are able to cleave the IL-6R, the metalloproteases ADAM10 and ADAM17 appear to be the most important enzymes involved in this process [18, 29-31]. Soluble forms of the IL-11R (sIL-11R) have not been described to date, although transcripts potentially encoding sIL-11R have been found [32]. However, experiments with recombinant proteins clearly showed that sIL-11R can bind IL-11, and the sIL-11R/IL-11 complex has agonistic properties that stimulated cell proliferation via gp130 [33-35]. Conflicting data exist whether sIL-11R can also act as an IL-11 antagonist on cells that express membrane-bound IL-11R [33, 36].

In conclusion, although signaling of IL-6 and IL-11 appears to be similar in several aspects, subtle differences in their biochemical properties might explain the different biological functions of these two cytokines *in vivo*.

Conflicting interests

The authors have declared that no conflict of interests exists.

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Author contributions

JL and CG wrote and approved the final manuscript.

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