Control and consequences of IL-6 receptor ectodomain shedding

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Background
Interleukin-6 type cytokines are mainly involved in inflammation by controlling differentiation, proliferation, migration, and apoptosis of target cells. A dysfunction of the complex regulatory cytokine network might lead to acute and chronic inflammation, autoimmune diseases or neoplastic disorders. IL-6 deficient mice were found to be resistant to collagen- and antigen-induced arthritis, highlighting the role of IL-6 in chronic inflammation and autoimmune diseases. The IL-6 receptor complex consists of the signal-transducing gp130 receptor and the non-signaling IL-6 receptor (IL-6R) which exists in membrane bound and soluble forms. A soluble form of the human IL-6R (sIL-6R) is mainly generated by limited proteolysis (ectodomain shedding) but also by alternative splicing. IL-6 signaling via the membrane-bound IL-6R and gp130 is called classic signaling, whereas IL-6 signaling via the soluble IL-6R and gp130 is referred to as IL-6 trans-signaling. A high concentration of 25–50 ng/ml sIL-6R is found in the serum of healthy humans. Under pathophysiological conditions, sIL-6R levels rise up to three fold. The cellular source of the sIL-6R and the in vivo mechanism of its generation are still unclear. The vast majority (90–99%) of the sIL-6R should originate from ectodomain shedding of the membrane-bound precursor, whereas alternative splicing of the IL-6R mRNA is accounts only for a minor proportion (1–10%) [1].

The sIL-6R is generated by constitutive and induced shedding by the “A disintegrin and metalloproteinases” (ADAM)10 and ADAM17 [1]. Recently, we characterized the structural requirements of IL-6R shedding on the site of the substrate [2]. The IL-6R consists of three extracellular domains, important for efficient exocytosis and IL-6 binding. The extracellular domains are followed by a flexible, 52 amino acids long stalk region, a trans-membrane domain and an intracellular domain which regulate basolateral sorting of the IL-6R [3]. The ADAM17 cleavage site was identified within the stalk region between the amino acids 357Q and 358D [1]. Deletion of 10 amino acids surrounding the ADAM17 cleavage site abrogated ADAM17 shedding of the IL-6R but leaves ADAM10 shedding intact. This suggested that the ADAM10 cleavage site is located at a different site. Interestingly, ADAM proteases do not have defined cleavage consensus sequences, which hinder the identification of novel cleavage sites by substrate sequence analysis. Deletion of 5 additional juxtamembrane located
amino acids in the aforementioned delta10 IL-6R variant abolishes also ADAM10-mediated ectodomain shedding of the IL-6R [2], suggesting that the ADAM10 cleavage site is in close proximity to the ADAM17 cleavage site. Importantly, both IL-6R variants (delta10 and delta15) are biologically active. Moreover, we showed that only about 20 of the 52 amino acids of the IL-6R stalk region are needed for biological activity of the IL-6R, which supported a model of the IL-6/IL-6R/gp130 signal transducing complex, in which the gp130 receptor chain has a C-shaped structure after IL-6/IL-6R/gp130 complex formation [2]. In this model, the stalk region is necessary for the correct positioning of the IL-6-binding domains of the IL-6R in the signal transducing receptor complex.

**Constitutive shedding is dependent on the level of cellular IL-6R expression**

Apart from induced IL-6R shedding, which is activated by various substances and conditions, including phorbol esters such as phorbol-12-myristat-13-acetate (PMA), the Ca2+ ionophor ionomycin, extracellular ATP, low membrane-cholesterol levels and apoptosis [1], the IL-6R is also constitutively released from the cell surface, without obvious cellular stimulation. Here, we demonstrated that cellular senescence and EGF-R stimulation lead to increased IL-6R expression, which was regulated via the mTOR pathway [4]. The simple increase in cell surface expressed IL-6R also led to increased generation of sIL-6R, suggesting that the expression level of the cellular substrate indirectly determines the amount of constitutive shedding. sIL-6R serum levels are increased under various inflammatory conditions and up to now, it is not clear if this is dependent on increased IL-6R expression, induced or constitutive shedding. Or data, however, open up the possibility that both mechanisms contribute to inflammation-induced increased sIL-6R levels.

**Conclusions**

In recent years, we characterized the biological functions of IL-6 trans-signaling and sgp130, which specifically suppresses overshooting IL-6 trans-signaling activities but to leaves “beneficial” IL-6 classic signaling intact. The biological sgp130Fc is currently tested in phase I clinical trials [5]. In contrast to treatment with neutralizing TNFalpha antibodies, administration of the trans-signaling inhibitor sgp130 did not interfere with protective immune responses after infection with *Mycobacterium tuberculosis* in mice [3]. Moreover, classical signaling was sufficient for early control of *Listeria monocytogenes* infection in mice [6]. Blockade of IL-6 trans-signaling might therefore exhibit advantages as compared to the global blockade of IL-6 or TNFalpha by monoclonal antibodies for the treatment of chronic inflammatory diseases.

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**References**

1. Scheller J, Chalaris A, Garbers C, Rose-John S: ADAM17: a molecular switch controlling inflammatory and regenerative responses. *Trends Immunol* 2011, 32:380-387.
2. Baran P, Nitz R, Grotzinger J, Scheller J, Garbers C: Minimal interleukin 6 (IL-6) receptor stalk composition for IL-6 receptor shedding and IL-6 classic signalling. *J Biol Chem* 2013, 288:14756-14768.
3. Scheller J, Garbers C, Rose-John S: Interleukin-6: From basic biology to selective blockade of pro-inflammatory activities. *Semin Immunol* 2013, 26:2-12.
4. Garbers C, Kuck F, Aparicio-Siegmund S, Konzak K, Kessenbrock M, Sommerfeld A, Haussinger D, Lang PA, Brenner D, Mak TW, Rose-John S, Essmann F, Schülze-Osthoff K, Piekarz RR, Scheller J: Cellular senescence or EGFR signaling induces Interleukin 6 (IL-6) receptor expression controlled by mammalian target of rapamycin (mTOR). *Cell Cycle* 2013, 12:3421-3432.
5. Jones SA, Scheller J, Rose-John S: Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *J Clin Invest* 2011, 121:3375-3383.
6. Hoge J, Yan I, Jänner N, Schumacher V, Chalaris A, Steinmetz OM, Engel DR, Scheller J, Rose-John S, Mittrucker HW: IL-6 controls the innate immune response against *Listeria monocytogenes* via classical IL-6 signaling. *J Immunol* 2013, 190:703-711.

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