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11

The soluble interleukin-6 receptor and related proteins

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Interleukin-6 is a cytokine involved in the regulation of the immune system and the central nervous system. Interleukin-6 binds to an interleukin-6 receptor, and then associates with a dimer of the ubiquitously expressed gp130 receptor subunit, which initiates intracellular signaling. The interleukin-6 receptor is found in a soluble form, which is generated by proteolytic cleavage and also to a minor extent by translation from an alternatively spliced mRNA. The complex of interleukin-6 bound to the interleukin-6 receptor can stimulate cells, which only express gp130. Such cells are not responsive to interleukin-6 alone. We have for the first time identified the molecular basis of pro-and anti-inflammatory properties of interleukin-6 and we have defined the generation of the soluble IL-6R as a crucial point in the regulation between these two properties. Furthermore, we have deduced a therapeutic principle, which enables us to exclusively block the pro-inflammatory activities of this important cytokine.

Introduction

Interleukin-6 (IL-6) is a four helical cytokine, which plays an important role in the coordination of the innate and acquired immune response [1] and is the major inducer of the hepatic acute phase

Abbreviations: CNTF, ciliary neurotrophic factor; EGF, epidermal growth factor; Fc, constant portion of an IgG antibody; GFAP, glial fibrillary acidic protein; GMP, good manufacture practice; IFN, Interferon; IL, interleukin; LIF, leukemia inhibitory factor; R, receptor; SNP, single nucleotide polymorphism; s, soluble; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor.

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response [2]. More recently it was established that IL-6 is essential for the regulation of the balance between regulatory T-cells and TH17 cells [3]. Interestingly, IL-6 also acts in the central nervous system on neurons and glial cells [4].

On target cells IL-6 binds to an 80 kDa IL-6 receptor (IL-6R). The complex of IL-6 and IL-6R associates with a 130 kDa receptor protein called gp130 [5]. The gp130 receptor thereupon dimerizes and initiates intracellular signal transduction by activating the JAK/STAT and ras/MAP kinase pathways [6]. While gp130 is expressed on all cells of the human body, IL-6R is mainly found on hepatocytes, some leukocytes and epithelial cells [7]. Interestingly, gp130 is also part of the receptor complexes of 8 additional cytokines, which form the family of IL-6 type cytokine [8].

Generation of the soluble IL-6R

A soluble form of the human IL-6R was first purified and identified in human urine [9] although at that time no function could be ascribed to this protein. Years later an alternatively spliced mRNA coding for a soluble form of the IL-6R (sIL- 6R) was detected. In this mRNA the exon coding for the transmembrane domain of the IL-6R is spliced out and reading frame is changed resulting in the translation of an additional 10 amino acids at the COOH terminus of the sIL-6R [10,11]. An antibody recognizing the altered COOH terminus of the alternatively spliced sIL-6R could be used to detect the sIL-6R protein generated by alternative splicing [12].

At the same time it was found that the human IL-6R was also proteolytically processed leading to the appearance of a 55 kDa soluble protein in cellular supernatants, which was recognized by an IL-6R specific antibody [13,14]. Remarkably, cellular IL-6R was completely released within 24 h and this release could be dramatically accelerated by stimulation of cells with the phorbol ester phorbol 12-myristate 13-acetate (PMA) leading to complete cleavage of the IL-6R within one hour [13,14].

Subsequently a cleavage site between glutamine and aspartate of the sIL-6R was determined to be in the extracellular portion of the receptor in close proximity to the transmembrane region of the protein [15]. Later it was shown that the IL-6R found in the human circulation was predominantly derived from proteolytic cleavage and only to a minor extent from alternative splicing [16,17]. Intriguingly, the alternatively spliced mRNA coding for a soluble IL-6R has only been found in humans but not in the mouse [10,12] whereas proteolytic processing of the IL-6R protein occurs in all species analyzed so far [13,18].

A Disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) is a membrane bound metalloprotease, which has been isolated as the enzyme responsible for releasing soluble TNFα from its membrane bound pro-form [19]. The enzyme contains a disintegrin domain, which is also found in many snake venoms [20]. Using inhibitors and mouse embryonic fibroblasts deficient for ADAM17 [21] it was shown that this enzyme is a major sheddase for the human IL-6R [22]. Also many ligands of the EGF-R, which are all synthesized as transmembrane precursors, are cleaved by ADAM17 [23]. Interestingly, ADAM17 has more than 70 known protein substrates including cytokines, cytokine receptors and adhesion molecules and the protease is involved in many pathophysiologic processes such as inflammation and tumor growth [24].

The function of the sIL-6R

It was observed by Taga et al. that the human IL-6R in the presence of IL-6 associated with gp130 to induce signaling [25]. Surprisingly, neither the cytoplasmic portion nor the transmembrane region of the human IL-6R was required to associate with gp130 [25]. Subsequently it was shown that the sIL-6R proteolytically cleaved from cells bound IL-6 and in complex with IL-6 could activate gp130 on cells, which did not express the IL-6R [13]. Therefore we hypothesized that cells with no IL-6R expression would respond to IL-6 in a sIL-6R dependent manner. We named this signaling pathway IL-6 trans-signaling (Fig. 1) [11].

To demonstrate that IL-6 trans-signaling was relevant in vivo we generated transgenic mice over-expressing the sIL-6R from the liver specific PEPCK promoter [26]. Since murine IL-6 does not bind to the human IL-6R the mice had no overt phenotype. However, when the mice were injected with human IL-6 it turned out that the sIL-6R drastically prolonged the half-life of IL-6 and led to a significant