REVIEW ARTICLES

DOI: 10.5281/zenodo.1299041
UDC: 577.112.6

A modern approach to interleukin-6

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Abstract

Background: Interleukin-6 is a multifunctional cytokine with well-defined pro- and anti-inflammatory properties. Binding to the receptor complex composed of specific interleukin-6-receptor (IL-6R) and transmembrane glycoprotein gp130, it stimulates various signalling cascades. Intracellular signal transduction involves both STAT-dependent, and STAT-independent mechanisms. IL-6R exists in the soluble and the membrane-bound forms, thus were described the classical signalling and the trans-signalling pathways. The pro-inflammatory response occurs via trans-signalling, while the anti-inflammatory effects are mediated by classical signalling pathway. IL-6 is produced by cardiovascular components. The high levels of IL-6 are identified in the inflammatory diseases.

Conclusions: IL-6 expresses the well-defined pro- and anti-inflammatory properties. The experimental studies have revealed classic signalling pathway with regenerative and anti-inflammatory effects (via the membrane-bound IL-6R), and trans-signalling responsible for the pro-inflammatory response (via the soluble form sIL-6R). The intracellular signal transduction involves the activation of STATs, MAPK, and PI3K cascades. It has been proved that in the cardiovascular pathologies the serum levels of IL-6 correlate with the disease severity and the degree of myocardial damage, being the indicator of heart disease and a predictive factor of adverse outcomes.

Key words: interleukin-6, cytokine, trans-signalling, cardiac ischemia.

Introduction

Cytokines are glycosylated proteins involved in the intercellular communication. The interleukin-6 (IL-6) type of cytokines participates in the inflammation, regulates the target cells differentiation, proliferation, migration and apoptosis [1], and is a key factor in coordinating innate and acquired immune response [2].

Its biological effect is achieved by homo- or heterodimerisation of glycoprotein 130 (gp130) [3]. The transduction of IL-6 type cytokine signal involves the activation of Janus (Jak) kinases, followed by the phosphorylation of transcription factors STAT [4], as well as triggering MAPK and PI3K signalling pathways [5]. The deregulation of both the IL-6-mediated signal transduction mechanism, and the intercellular communication processes generates neoplasms, severe autoimmune and inflammatory diseases [6].

Interleukin-6 (IL-6) is a small glycoprotein that activates the cells via the heterodimeric signalling complex, consisting of the alpha receptor (IL-6R) and the beta subunit for signal transduction, represented by glycoprotein 130 (gp130) common to all cytokines type IL-6 [7].

IL-6-mRNA encodes the 212 amino acid protein, including the 29 amino acid signalling peptide. The secreted protein contains 184 amino acids (21 kDa) [8], of which 107 are well defined in the final structure, while 18 N-end amino acids and 8 amino acids from AB loop have no visible electron density [9]. There have been described the different 21-28 kDa isoforms, formed due to the various N-linked glycosylation, which determines the stability and half-life of the protein [10]. IL-6 is synthesized and secreted by the most cells, including T cells, fibroblasts, monocytes, and endotheliocytes.

The IL-6 conformation consists of 4 long alpha-helixes (A, B, C, D) [8], with the typical "up-up-down-down" arrangement, that is common to all IL-6 type cytokines [2]. The attachment to the IL-6R / gp130 receptor complex takes place through three well-differentiated contact sites: **site 1* (binds IL-6R), **site 2* (attaches gp130 between the domains 2 and 3), and **site* 3 (contacts the immunoglobulin-like domain 1 of gp130) [2]. The *site 1* is formed by the C-end groups of D-helix and the C-end part of the AB loop, and determines the specificity of binding to IL-6R. The *site 2*, consisting of the middle sequences of the A and C helixes, and the *site 3*, formed by the N-terminal part of the AB loop (3a) and the C-end of the D helix (site 3b), are required for the recruitment of 2 molecules of gp130 [2].

The synthesis of IL-6-mRNA is regulated at both the transcriptional, and the post-transcriptional levels. The key role in IL-6 expression is attributed to nuclear transcription factor kappa B (NF-κB), activated by the bacterial lipopolysaccharides, pro-inflammatory cytokines (TNFα) or viruses [11].

Experimental studies have highlighted fact that a large number of microRNAs (miR) inhibit IL-6 expression, in-

cluding miR-26a [12], miR-142 [13], miR-146a [14], miR-187 [15], miR-200S [16], miR-329 [17].

Very low amounts of IL-6 (about 1-5 μg/ml) there are in the blood of healthy people. The high serum levels of IL-6 are identified in the most of inflammatory and/or autoimmune diseases, reaching the μg/ml values in septic states [18]. IL-6 is the most potent activator of the synthesis of the acute phase proteins in hepatocytes, including the C-reactive protein, and it is an important factor for tumor growth [19]. Kishimoto T. (2010) has suggested that IL-6 is a marker of the continuous inflammation [20].

IL-6 is also produced by cardiovascular components, such as endothelial cells, vascular smooth muscle and ischemic cardiomyocytes [21]. The expression of IL-6 is stimulated by the C-reactive protein via the nuclear factor B (NF-B), and inhibited by the nitric oxide (NO). It was proved the involvement of IL-6 in the cardiac metabolism regulation [22]. Yudkin J. et al. (2000) have shown the role of IL-6 in the pathogenesis and clinical development of atherosclerotic vascular lesions [23].

The high levels of circulating IL-6 are associated with the increased risk of mortality and poor clinical outcome in the patients with unstable angina [24]. Clinically was confirmed that the IL-6 concentrations not only correlate with the severity of the disease, but are also the important predictors of adverse outcomes. It has been shown that the pro-inflammatory cytokines participate in the destabilization and disruption of the atherosclerotic plaque in the coronary arteries, by stimulating the expression of matrix metalloprotease that are responsible for the vascular remodelling and the plaque disorganization [25].

The increased expression of myocardial IL-6 is associated with the progression of heart failure, so IL-6 can be a true indicator of heart damage [26]. The experimental data show evidence that pro-inflammatory cytokines can depress myocardial contractility and are responsible for the cardiovascular disease development and evolution [22]. In the patients with acute coronary syndrome the serum levels of IL-6 correlate with the severity of myocardial lesions [27].

IL-6 is involved in the control of vascular permeability by stimulating the production by the fibroblasts of the vascular endothelial growth factor (VEGF) that acts on the endothelial cells, as well as by enhanced collagen synthesis [28]. There has been identified the association of IL-6 with endothelial cell activation markers, cell adhesion molecules (VCAM1, ICAM1) and von Willebrandt factor [29]. In 2013 Zamani P. et al. (2013) have confirmed that elevated levels of VCAM1 correlate with the increased risk of coronary events in patients with acute coronary syndrome [27].

The published data have proved that IL-6 contributes to the resorption of acute neutrophil infiltration by inducing apoptosis of neutrophils [30]; while the T-cell apoptosis is prevented by activating the STAT3-dependent anti-apoptotic factors (Bcl2, Bclx1) [31].

The *receptor complex* that mediates the biological response of IL-6 consists of the transmembrane glycoprotein type I for IL-6 binding, called alpha-IL-6R (CD126 or gp80), and transmembrane protein type I for signal transduction (beta-subunit, CD130 or gp130) [2]. The expression of gp130 is characteristic of all body cell types, whereas the expression of αIL-6R has a limited character and is characteristic of hepatocytes, megakaryocytes, and some leukocyte subpopulations (monocytes, macrophages, B and T cells) [32].

The experimental functional and structural studies suggest that IL-6 can form both the hexameric, composed of 2 molecules of IL-6, IL-6R and gp130 ($IL\text{-}6_2/IL\text{-}6R_2/gp130_2$) [33], and the tetrameric ($IL\text{-}6/IL\text{-}6R/gp130_2$) signalling complexes [34]. Viswanathan S. et al. (2002) have shown that the low IL-6 concentrations favour the formation of the tetrameric complexes, whereas the high concentrations of IL-6 will lead to the formation of the hexameric complexes [35].

Interleukin-6 receptor (IL-6R) represents the 80 kDa glycosylated membrane protein [36]. The immunoglobulin-like domain of the human IL-6R does not participate in the IL-6 binding, but it is responsible for the receptor stability. The attachment of IL-6 to IL-6R is mediated by the specific sequences located in the domains 2 and 3 of IL-6R [2].

IL-6R exists in both the soluble and the membrane-bound forms, which allow differentiating the classical signalling pathway (via the membrane-bound IL-6R) and the trans-signalling (through soluble (sIL-6R) receptor). In 2012 in his studies, Rose-John S. mentioned that the trans-signalling pathway represents the pro-inflammatory part of the IL-6 biological effect [8], while the classical signalling exhibits the anti-inflammatory and regenerative activity [1]. The experimental studies confirmed that the regenerative, protective and anti-inflammatory effects of IL-6 [37] are mediated through the membrane-bound IL-6R, which is responsible for the differentiation of pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages [38]. Also via the classical signalling pathway IL-6 is involved in the hepatic regulation of the insulin sensitivity and glucose tolerance [39].

Wilke C. et al. in 2011 highlighted the role of trans-signalling in the immune system adaptation, and the T cells recruitment, activation and apoptosis [40]. The target cells for trans-signalling are the stem cells: hematopoietic [41], nerve [42], smooth muscle [43], and embryonic [44]. The trans-signalling ensures the migration of the lymphocytes in the inflammation area [45]; it induces T cell proliferation and participates in the regulation of the adhesion cells expression in the endothelial cells [46].

IL-6R can be cleaved proteolyticaly from the cell membrane surface and produce the *soluble form of IL-6R (sIL-6R)* [47]. Taga T. et al. (1989) have demonstrated that the cytosolic region and the transmembrane domain of IL-6 do not participate in the signalling mechanisms, so they have hypothesized that the formation of sIL-6R is a tool for in-

volving the cells that possess only gp130 in the signalling pathways mediated by IL-6 [48].

The soluble sIL-6R receptor is generated by two different mechanisms: * the shedding of the membrane-bound receptor (90-99%) [2, 47]; * the alternative splicing (approximately 1-10%) and the transcription of IL-6R-mRNA omitting the exon 10 that encodes the transmembrane and cytosolic domains [47, 49]. The proteolytic cleavage of IL-6R is catalysed by the ADAM-10 and ADAM-17, Zn^{2+} metalloproteases (sheddases) [2, 50], the transmembrane proteins involved in the partial proteolysis of the membrane receptors of the cytokines [51]. The cleavage site for ADAM-17 is located between Gln357 and Asp358 in the region adjacent to cell membrane [52], the complete cleavage of IL-6R from the cell surface occurs within 24 hours [47]. It was supposed that ADAM-10 is responsible for the slow cleavage, whereas ADAM-17 favours the rapid proteolysis of the membrane-bound IL-6R [50]. In addition to the ADAM family of sheddases, the IL-6R also can be cleaved by the cathepsin G [53].

The biological activators of ADAM-17 are the pro-inflammatory cytokines (TNFα) [54], reactive C protein, the bacterial toxins (streptolysin O, hemophilin A) and metalloproteases, as well as the apoptotic pathways [55]. In 2011 Scheller J. et al. have mentioned the decisive role of ADAM-17 in the cancer genesis and inflammation [56]. The published data demonstrated that the apoptosis-induced cleavage of IL-6R is caspase-dependent, and PKC/MAPK/ROS independent [2].

According to McFarland-Mancini M. et al. (2010) the main sources of sIL-6R are the hepatocytes and hematopoietic cells, as well as the immune cells (neutrophils, macrophages) [57]. It has been hypothesized that the soluble forms of the receptor are the important regulators of the inflammatory processes [2].

The recent studies have found that the single base pair polymorphism determines the serum level of sIL-6R in humans. The substitution of adenine with cytosine leads to the replacement of Asp358 with Ala358 in the exon 9 of the IL-6 gene [58]. In some studies it has been mentioned the association of Asp358Ala variant with low risk of coronary events [59].

According to Scheller J. et al. (2011) the *glycoprotein 130 (gp130)* is a membrane type I glycosylated protein with a molecular weight of 130-150 kDa, consisting of 6 extracellular domains, one transmembrane domain and one cytosolic domain. The N-terminus has the immunoglobulin-like domain (D1), followed by 2 cytokine binding domains (CBD, the domains 2 and 3) and 3 fibronectin (FN-III) domains (the domains 4-6) [2].

In 2003 Boulanger M. et al. demonstrated that the immunoglobulin-like domain of gp130 ensures the interaction with IL-6R and the attachment to the IL-6 site 3 [35]. The cytokine binding domains (CBD2 and CBD3) of gp130 associate with IL-6 site 2. CBD2 located in the N-end region contains 2 pairs of Cys, which form inter-chain disulphide bonds, while CBD3 contains the Trp-Ser-X-Trp-Ser sequence [60]. The proximal membrane domains of gp130 are involved in the signal transduction to the cytosolic domain [2]. After the ligand binding, the domains 4 and 5 are rearranged at an angle of 80° with the curve formation, thus the whole gp130 ectodomain acquires the structural conformation similar to a wide open «C» [61].

The receptor gp130 subunit has affinity for neither IL-6 nor IL-6R [62]. Therefore, IL-6 can only fix and stimulate the cells that possess IL-6R, while the cells that have only gp130 are completely irresponsive to the IL-6 cytokine signals [63].

The myocardial ischemia/reperfusion induces the expression of gp130, associated with the stimulation of IL-6 and IL-6R expression, thus confirming the role of the IL-6/IL-6R/gp130 system in acute myocardial infarction [12].

Gp130 serves as the β-subunit of the receptor common for the IL-6 cytokine family. Recently have been described several *soluble molecules (sgp130)* with lower molecular mass [64], generated by: (1) shedding, (2) alternative splicing, (3) the addition of an exon consisting of 85 base pairs that alters the codons reading by forming a STOP-codon prior to the transmembrane domain coding sequence, (4) the deletion of an exon with new C-terminal sequence (NIASF) formation, followed by STOP-codon, or (5) the alternative polyadenylation of the intron 10 resulting in new mRNA [65].

Due to the fact that the secreted IL-6 binds in the plasma with sIL-6R, and with sgp130, it has been experimentally proved that the soluble form of gp130 (sgp130) inhibits the trans-signalling pathway [66]. Jostok T. et al. (2001) have confirmed that sgp130 interacts only with sIL-6R in the presence of IL-6. The balance between the soluble and membrane forms of gp130 plays an important role in regulating the biological effects of cytokines. When the amount of IL-6 exceeds the amount of sIL-6R and sgp130, IL-6 will act systemically [62].

The recent publications have demonstrated that the single base pair polymorphism in the IL-6R gene manifested by the replacement of Gln258 with Ala, results in higher serum concentrations of sIL-6R, and is associated with reduced risk of coronary heart disease [67]. Boekholdt S. and Stroes E. (2012) have determined that the cleavage of membrane-bound IL-6R on the surface of hepatocytes, monocytes and macrophages results in the loss of cell sensitivity to IL-6 mediated signals [68]. Scheller J. and Rose-John S. (2012) have assumed that high serum levels of sIL-6R increase the buffer capacity of the sIL-6R/sgp130 complex in the blood, thereby diminishing the systemic effects of IL-6 [69].

There has been shown that the single base pair polymorphism (G148R) in gp130 deregulates the function and stability of the protein, and it is associated with a low risk of acute myocardial infarction [70]. Elevated serum levels of sgp130 were identified in patients with heart failure, being associated with higher mortality rates. In 2007 Ichiki T. et al.

presented the data that in the patients with acute myocardial infarction the serum levels of sgp130 were the highest at admission, and were followed by the decreasing in the post-infarction period [71]. At the same time, the patients with progressive heart failure have higher levels of sgp130 compared to the patients with stable heart failure, so the sgp130 can be used to identify patients at high risk for the heart failure progression [72].

Both the experimental animal studies, and in vitro modelling, have highlighted the beneficial effect of sgp130 in atherosclerosis as result of the trans-signalling pathway inhibition by the specific binding of the IL-6/sIL-6R heterodimer [62, 73]. It has been assumed that the higher levels of sgp130 represent the compensatory response to increased IL-6 signalling in chronic ischemic disease and vascular remodelling [73]. Moreno Velasquez I. et al. (2015) have shown the association of elevated serum levels of sgp130 with a 30% reduction in acute myocardial infarction incidence [74]. There was confirmed the hypothesis that in acute coronary syndromes the sgp130 changes correlate negatively with the severity of the inflammation [75]. The published results have suggested that sgp130 may be used as the prognostic biomarker in cardiac diseases [76].

The classical *signalling pathway* starts with IL-6 attachment to the membrane receptor αIL-6R, the formation of the signalling complex with the gp130 homodimer, the signal transduction and activation of the intracellular signalling pathways [2]. The cells that express only gp130 can be stimulated by the IL-6/sIL-6R complex via the trans-signalling pathway [77].

Cellular response to IL-6/sIL-6R complex differs substantially from the response to IL-6. This can be explained by: * the cell expresses more gp130 than IL-6; ** IL-6 is internalized faster compared to the IL-6/sIL-6R complex [78]. Experimentally has been demonstrated that the IL-6 affinity for IL-6R is 1nM, whereas the affinity of IL-6/sIL-6R complex for gp130 is 100-fold greater [79].

The gp130 cytokine receptor does not possess intrinsic kinase activity. The first step in the intracellular transduction of the IL-6 signal is the activation of Janus (Jak) kinases (Jak1, Jak2 and Tyk2), that are non-covalently constitutive associated with the cytosolic tail of gp130. FERM domain ensures the interaction of Jak-kinases with gp130 [80]. The IL-6 binding to the specific receptor favours the induction of Jak-kinases by the tyrosine sequence phosphorylation, located on the activation loop of C-terminal JH1 domain. The active Jak-kinases lead to phosphorylation of the distal tyrosine residues from the cytosolic part of gp130, which represent the recruitment sites for STAT (signal transductor and activator of transcription) factor. STAT1 or STAT3 monomers attach to the distal phosphotyrozine residue, the tyrosine phosphorylation of STAT occurs, followed by homo- or heterodimerisation, the translocation of STAT dimers in the nucleus, N- and C-terminal acetylation, CBP/p300 interaction, and the attachment to the promotor region of IL-6 inducible gene [81]. Thus, the cytokines binding will induce the reorganization of the preformed receptor complexes and the successive cross-phosphorylation [2].

IL-6 activates the cascades STATs, MAPK, and PI3K [82]. The balance between activation of STAT3 and MAPK is crucial for the controlled proliferation of cells, and ultimately for the body's homeostasis [83]. This balance is regulated by the cytosolic Tyr759 of gp130; the tyrosine phosphorylation will reduce IL-6-dependent STAT3 activation [84], and induce MAPK activation [85]. At the same time, the phosphorylation of the gp130 Tyr759 residue is essential for the membrane IL-6-dependent recruitment of Gab-1 protein, which acts as the bridge between the cascades MAPK, and PI3K, and the activated receptor complex [86]. Chen R. et al. (1999) have concluded that the survival mechanisms are maintained via the activation of PI3K and Akt [87].

Conclusions

Interleukin-6 expresses the well-defined pro- and anti-inflammatory properties. The experimental studies have revealed classic signalling pathway with regenerative and anti-inflammatory effects (via the membrane-bound IL-6R), and trans-signalling responsible for the pro-inflammatory response (via the soluble form sIL-6R). The intracellular signal transduction involves the activation of STATs, MAPK, and PI3K cascades. It has been proved that in the cardiovascular pathologies the serum levels of IL-6 correlate with the disease severity and the degree of myocardial damage, being the indicator of heart disease and a predictive factor of adverse outcomes.

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