ISSN 0006-2979, Biochemistry (Moscow), 2011, Vol. 76, No. 4, pp. 394-406. © Pleiades Publishing, Ltd., 2011.
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REVIEW

# Functional Properties of Extracellular Domains of Transducer Receptor gp130

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Received August 6, 2010
Revision received September 17, 2010

**Abstract**—Cytokine receptor molecules have been shown to have extracellular domains of complex structure and a multistep activation system. Glycoprotein gp130 is a typical transducer of cytokine signal; it functions by forming multicomponent receptor complexes and transferring signals of tens of cytokines from the IL-6 family. Structural organization and basic functioning principles of gp130 are well known, as well as related signal pathways, which function during normal differentiation and are involved in pathogenesis of many tumors. The role of gp130 in IL-6-dependent tumors is best studied. In this review, based on extensive accumulated data, we examine the functional significance of certain parts of gp130 extracellular domains. Potentials of a recently developed method for estimation of receptor activation at the level of epitope structure are discussed.

**DOI**: 10.1134/S000629791104002X

*Key words*: gp130 activation, cytokine, immunophenotyping, monoclonal antibodies, receptor structure, IL-6, cytokine-binding homology region

Receptor systems that require the gp130 glycoprotein for their functioning mediate the transduction of tens of cytokines from the IL-6 family or gp130-cytokines. The list of these cytokines is constantly expanding and now contains IL-6, IL-11, LIF, OSM, CT-1, CNTF, IL-27, CLC, NPN, HHV8 IL-6, and Rm IL-6. The process of transduction involves the activation of intracellular signaling JAK/STAT, Ras/MAP, and PI3K/AKT pathways and the modulation of apoptotic processes and cell growth and differentiation [1-4]. Ubiquitous expression of gp130 in human cells and the diversity of biological processes mediated by it are reflected by the complexity of mechanisms of cytokine reception, the high level of pathogenic involvement, and, clearly by the attractiveness of gp130 as an object for molecular studies.

Intracellular mechanisms of cytokine signal transduction mediated by gp130 have been reviewed in detail [5-7]. Glycoprotein gp130 does not have its own kinase domain, but its cytoplasmic part is associated with JAK kinases. In the activated (dimerized) state it undergoes phosphorylation of its six tyrosine residues, each of which is responsible for further direction of the signaling cascade [8, 9]. Depending on the nature of the acting cytokine, the type and physiological state of cells, gp130 participates in the regulation of immune response, haemopoiesis, acute phase response, endocrine and neuropoietic processes, heart muscle development, etc. [10-12]. Thus, a gp130 molecule may act as a therapeutic target under the abnormalities in these processes, given precise action on specific cytokine binding sites [13, 14]. According to the international nomenclature of human leukocyte differentia-

*Abbreviations*: CHR, cytokine-binding homology region; CLC, cardiotrophin-like cytokine; CNTF, ciliary neurotrophic factor; CNTFRα, ciliary neurotrophic factor receptor; CT-1, cardiotrophin-1; ELISA, enzyme-linked immunosorbent assay; HHV8 IL-6, interleukin-6 from human herpes virus type 8 genome; IgD, immunoglobulin-like domain; IL-6, -11, -27, interleukin 6, 11, 27; IL-6Rα, interleukin-6 receptor; JAK/STAT, signal cascade with participation of Janus-kinases (*JAK*) and signal transducers and activators of transcription (STAT); LIF, leukemia inhibitory factor; LIFR (gp190), leukemia inhibitory factor receptor; mAbs, monoclonal antibodies; NPN, neuropoietin; OSM, oncostatin M; PI3K/AKT, signal cascade with participation of phosphatidylinositol 3-kinase and protein kinase B (AKT); Ras/MAP, signal cascade with participation of membrane-bound GTPase Ras and mitogen-activating kinases (MAP); Rm IL-6, interleukin-6 from rhesus macaque adenovirus genome; SOCS, suppressor of cytokine signaling proteins.

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tion antigens, gp130 is labeled as CD130, and its expression is most prominent in T-cells, activated B-lymphocytes, plasmocytes, monocytes, and endotheliocytes.

Processes occurring on the cell surface when cytokines act on gp130 are studied in less detail and are subject to long-standing discussion in the area of fundamental research of molecular mechanisms of signal transduction by type I cytokine receptors. This is due to the high complexity of the structure of the extracellular gp130 fragment and also to the diversity of cytokines and receptor systems involved in direct interaction with gp130. The gp130 molecule, being a common feature of many extracellular oligomeric receptor systems, which consist of cytokine-binding transmembrane and soluble primary receptors (α-chain) and cytokines proper, has the exclusive function of signal transmission into the cell. Considering the functional consequences of this principle of signal transduction, one should note pleiotropy (diversity of effects) and redundancy of cytokine functions [15]. Studies of recognizing and regulatory motifs of the gp130 extracellular part with specific monoclonal antibodies, site-directed mutagenesis, X-ray diffraction analysis, and computer simulations has made it possible to overview the mechanisms of action of important biological molecules from a broad family of gp130 cytokines and their receptors and explain and predict functional cytokine-mediated cell properties in various organs and tissues [16-18]. However, many fundamental questions about the mechanisms of reception and signal transduction by gp130 remain unresolved. These issues include mechanisms of selective action of certain cytokines in certain types of cells, principles of recognition of topologically and chemically different ligands by one receptor molecule, and systems of regulation of signal transduction. Answers to these questions lie in understanding the functional significance of gp130 extracellular domain structural features and detailed analysis of experimental data pertaining to gp130 signaling.

## STRUCTURE of gp130

The structure of the transducing gp130 molecule has been studied since the mid 1990s [19-21] and is now described in considerable detail [22]. Despite this, the Protein Data Bank (PDB) provides only five crystal structures involving the gp130 molecule: cytoplasmic domain phosphorylated in Y757 position in complex with SOCS3 (2HMH, 2BBU); hexamer formed by extracellular domains of gp130 D1, D2, and D3 with IL-6R and IL-6 (1D9M); tetramer of viral interleukin-6 and D2 and D3 domains of gp130 (1I1R), and LIF bound to D2 and D3 of gp130 (1PVH). The gp130 molecule (Fig. 1; see color insert) is a glycoprotein with molecular mass of 130 kDa with a polypeptide chain of 918 amino acid residues (a.a.) including cytoplasmic domain of 277 a.a., transmembrane domain of 22 a.a., and extracellular domain of 619 a.a. The *N*-terminal extracellular part of gp130 is formed by: i) distal to membrane immunoglobulin-like domain (D1, IgD); ii) cytokine-binding domain (CHR, cytokine-binding homology region) that is conservative for all receptors of hematopoietin receptor superfamily. It contains two fibronectin type-III domains (D2, D3), one of which (D2) is characterized by two cysteine bridges and the other (D3) by WSXWS motif, also conservative for this group of cytokine receptors, and iii) three other fibronectin type-III domains (D4, D5, D6). The native human gp130 molecule may be not only membrane-associated, but also be present in the extracellular space in soluble form. These glycoproteins with molecular mass of 100 kDa, in fact, lack transmembrane and intracellular parts of the gp130 molecule, which are formed as a result of proteolysis or alternative splicing according to various sources [23-25]. The presence of an alternative form of gp130 possessing cytokine-binding but not transducer properties can be interpreted as a manifestation of antagonistic regulation of signal transmission, but there are suggestions [26] that it might contain additional regulatory sites and can bind not only cytokine complexes with their α-chains, but also α-chains alone or other molecules by themselves. Recombinant gp130, which is the extracellular part of the transducer molecule, is produced in a baculovirus expression system in insect cells or expressed in CHO cells and isolated from the supracellular supernatant by immunoprecipitation on anti-gp130 monoclonal antibodies; it has a molecular mass of 65 kDa, is not a glycoprotein, but retains cytokine-binding properties [27-29].

## PRINCIPLES OF FORMATION OF OLIGOMERIC COMPLEXES OF gp130

**Cytokine binding sites.** During recent years a number of studies conducted at Stanford University by C. Garcia et al. [13, 30, 31] significantly added to existing data on the structure and functional properties of the gp130 extracellular domains. With crystallographic and calorimetric methods the thermodynamic and steric parameters of the interacting protein surfaces were described and compared, and the mechanisms of oligomeric gp130 receptor complex formation were predicted. Thus, the unique IgD domain discriminating gp130 from other hematopoietin receptors was shown to possess the ability to activate the signal transduction [31, 32], and assembly processes of multi-chain gp130 complexes go in discrete steps by binding to three conservative sites (Fig. 2; see color insert). Reception of IL-6 means that the cytokine initially binds to its α-receptor IL-6Rα in site I, forming a IL-6/IL-6Rα complex and preparing specific epitope for site II of gp130 (in CHR domains D2 and D3). Binding of IL-6/IL-6Rα to CHR leads to the formation

of epitope for site III in the ternary complex, and then IgD-domain (D1, site III) participates in the formation of an active hexamer. Multiple steps of binding also mean that two molecules of gp130 may be independently involved in the binding of one ligand [33]. If, as in the case of LIF, the formation of the complex occurs without the participation of α-receptor, the sequence of binding is determined by preferred affinity: LIF forms a strong bond with IgD-domain of LIFR homologous to gp130 (site III), and then with CHR domain of gp130 (site II) [13, 34]. These principles suggest some features of the assembly and structure of other cytokine gp130 complexes. The tetrameric architecture of gp130/LIFR/CNTFRα/CNTF complex, which obviously involves all three sites, is already confirmed [35].

**Principle of "thermodynamic plasticity".** The structural rigidity principle of the cytokine-binding surface of gp130 CHR domain was another important discovery, contrasting features of formation of gp130 oligomeric complexes with the common "plastic" mechanism, which has been established for a number of hematopoietic receptors [30, 36, 37]. This feature was consistently observed when describing the interactions of viral IL-6 with gp130 [31] and later of LIF with gp130 [13]. In all cases of complex formation, spatial conformation of the cytokine-binding surface of gp130 CHR domain remains unchanged, and the affinity for chemically and sterically diverse ligands is achieved by the amphipathicity of the majority of CHR amino acids (primary participation of either hydrophilic or hydrophobic amino acid residues in the binding in different cases) and by significant changes in their thermodynamic characteristics. This principle allows high affinity binding of a much wider range of molecules and is not limited by uniformity of the structure of their active centers. While the superimposition of the interaction surfaces of cytokines with gp130 CHR domain demonstrates dominant overlapping, the individual interactions of cytokines, which are observed at the CHR periphery, define the unique binding of the specific cytokine and may be regarded as structural design target for high-affinity cytokine antagonists [13].

## MONOCLONAL ANTIBODIES IN STUDIES OF gp130

Molecular studies of native receptors in living cells undoubtedly provide opportunities for a more versatile study of molecules and their complexes as compared with the description of computer models of recombinant molecule fragment structures. Intensive study of gp130 with the methodology of epitope mapping has been very informative and provided a dynamic view on the structural features of intricate receptor complexes with it. With the introduction of hybridoma technology for obtaining unique specific monoclonal antibodies that block the epitopes (antigenic determinants) of biological molecules into routine practice, it became possible to determine direct correspondence between certain elements of structure and their functional properties. This approach to the description of gp130 has been used by several groups [27, 29, 38, 39]; more than 60 functionally different monoclonal antibodies (mAbs) to the epitopes of the recombinant extracellular part of gp130 molecules have been studied, and antibodies were discussed at the VI International Human Leukocyte Differentiation Antigens Workshop (HLDA Workshop) [40, 41] and used in clinical and laboratory studies of haematopoiesis disorders [42, 43] and acute phase response [44]. Table 1 shows information about the monoclonal anti-gp130 antibodies and their impact on processes in cells mediated by cytokines of the IL-6 family.

**Functional effect of monoclonal antibodies and epitope mapping of gp130.** Blocking of different gp130 sites with antibodies, generally resulting in inhibited cytokine-stimulated cellular response, makes it easy to assign mAbs to groups corresponding to different functional cell responses to cytokines of the IL-6 family. This differentiation correlated to some extent with the spatial arrangement of epitopes responsible for specific cytokine signal transduction. However, the proximity of epitopes, similar mechanisms of gp130 activation by various cytokines, and type (Table 2) and cultivation conditions of cell cultures make the interpretation of experimental data ambiguous. In addition to the functional mapping of gp130 using the mAbs described above, the ability of antibodies to compete with each other for spatially close regions of the molecule have been studied [29, 38, 45]. Sandwich-ELISA methodology, involving consecutive fixation of anti-gp130 mAb (X) – gp130 – anti-gp130 mAb (Y) on the surface, revealed sterically competitive relations for all pairs of X–Y anti-gp130 mAbs when used *in vitro* (Fig. 3).

**Detection of gp130 activation states by flow cytometry.** A more thorough description of the mechanism of gp130 action with the use of mAbs was obtained using flow cytometry. The phenomenon of gp130/80 epitope redistribution under the influence of IL-6 was described in 1998 [42]. This makes it possible to identify the activated and inactivated states of gp130 and IL-6Rα on the basis of the receptor epitope structure [42]. The variety of binding epitopes and functional effects of various anti-gp130 mAbs allow us to study the stages of receptor complex formation prior to signal transmission into the cell and the sequential conformational changes of gp130 chains corresponding to them. In other words, using a set of mAbs made it possible to perform immunophenotyping of the extracellular part of the receptor in living cells [46]. Thus the antibodies of group A (Table 1), which prevent the effect of all cytokines, were shown [39] to directly bind with the site of gp130 dimerization. While the non-activated receptor is able to bind antibodies of group A, receptor dimerization occurs in the case of preincuba-

**Table 1.** Properties of anti-gp130 mAbs

| mAb | Function | Cell type | Reference | Identified and supposed domains of binding with gp130 |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| Common characteristic – inhibition of IL-6/IL-6R binding and of IL-6-induced proliferation | | | | |
| B1/C34 | agonist in pair with F1 or I2 (XG2) | XG1, XG2 [42, 51] | [39, 40, 42, 47, 51] | D1 |
| B2 | agonist in pair with I1 or C5 (XG2) | XG1, XG2 [42, 51], XG-4-CNTF [29] | [29, 39, 40, 42, 47, 51] | D1 |
| B3 | agonist in pair with I2 (XG2) | XG2 [51], XG-4-CNTF [29] | [29, 51] | D1 |
| B4 | | | | |
| B5 | agonist in pair with I2 (XG2) | XG2 [51], XG-4-CNTF [29] | [29, 51] | D1 |
| B-T2/C17 | inhibits action of IL-6, IL-11, CNTF, CT-1 [45], i.e. except LIF and OSM, binds with D1 [34] | XG2, TF-1 | [34, 45, 47] | D1 – [34] |
| B-T9/C18 | nonspecific – inhibits CNTF action | | [45] | |
| B-A32 | | | | |
| E1/C37 | Fab-fragments in pair with anti-mice Ig – agonists of proliferation | XG2 | [29, 39] | D1 |
| E2 | | | [29] | D1 |
| E3 | | | | D1 |
| 32F6 | specifically blocks IL-6-proliferation | | [45] | |
| B-P4/C20 | inhibits IL-11 and CNTF [38]-induced proliferation. Does not inhibit proliferation of SK-N-MC [29] | TF-1 [45], SK-N-MC [29] | [29, 38, 45] | D4 – [49] |
| Common characteristic – inhibition of OSM/LIF-induced proliferation | | | | |
| C1 | C1-C6 cannot inhibit IL-6-induced proliferation of XG2 cells [29] | | [29, 40, 47] | D3 |
| C2 | does not participate in IL-6 signal transmission | | [29, 40, 42, 47] | D3 |
| C3 | | | [29, 40, 47] | D3 |
| C4 | | | [29, 40, 47] | |
| C5 | agonist in pair with I1 (XG2 [51]) [39] | XG1, XG2 | [29, 40, 47] | D3 |
| C6 | | | [40, 47] | D3 |
| C7 | nonspecific, also inhibits action of IL-6 and CNTF [29] | | [29, 47] | D3 |

**Table 1.** (Contd.)

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| B-K5/C8 | competes with B-K11/C9, B-S12 [38], inhibits IL-6 effect [45], binds with OSMR type I and II [45] | TF-1 (OSMR I), A375 (OSMR II), HepG2 (OSMR I,II) | [38, 45, 47] | D3 |
| B-K11/C9 | specific to OSM [40, 45, 47]; nonspecific, also inhibits IL-6 and CNTF action in high concentrations [29], competes with B-S12 and B-K5 [38], displaced by IL-6/IL-6R complex [49] | Ba/F3-gp130 [52] XG-4-CNTF [29] | [38, 40, 29, 45, 47, 52] | D3 |
| B-L9/C10 | inactive [40] | XG-4-CNTF [29] | [29, 40, 47] | D3 |
| B-N4/C11 | nonspecifically inhibits action of IL-6 and weakly inhibits IL-11 [45]; inactive [40] | | [40, 45] | D3 |
| B-N9/C12 | in high concentrations inhibits all cytokines action [45]; specific to LIF/OSM [40, 47] | | [40, 45, 47] | D3 |
| B-R9/C13 | weak effect; inhibits effects of CNTF and CT-1 [45]; specific to OSM [47] | A375 | [45, 47] | D3 |
| B-S1/C14 | specific to OSM [47] | TF-1 [45] | [45, 47] | D3 |
| B-S12/C21 = B-S12-A5 + B-S12-G7 | competes with B-P8 [38] | | [38, 52] | D3 – [52] |
| AM64/C93 | masked by IL-6 in high concentration, demonstrates inhibiting effect after dexamethasone treatment; specific to LIF/OSM [47] | | [47] | |
| B-T12/C16 | blocks OSM, CT-1 effect in high concentration | TF-1 [45] | [45] | D4 |
| Common characteristic – inhibition of all cytokines action and especially CNTF-induced proliferation | | | | |
| F1 | agonist in pair with B1 (XG2 [51]); F1-F3 cannot inhibit IL-6-induced proliferation of XG2 cells [29], more specific to CNTF | XG-4-CNTF [29] | [29, 51] | D3 |
| F2/C38 | also inhibits LIF, OSM, and IL-6 effect and does not inhibit proliferation of IL-6-dependent XG2 cell line [29] | XG2, XG-4-CNTF [40] | [29, 40, 47] | D3 |
| F3 | | XG-4-CNTF [40] | [40, 47] | D3 |
| F4 | | XG-4-CNTF [40] | [40, 47] | D3 |
| B-P8/C19 | does not compete with B-S12 [38], specific towards CNTF [45], is analog of B-S12-A5; Fab demonstrates agonist action in pair with B-S12-G7; similar to B-P4 epitope (flow cytometry) [45] | TF-1 and SK-N-MC are not phosphorylated [45] | [38, 45, 52] | D3 – [38] |
| B-S12-A5 | overlapping with B-P8 epitope, Fab fragments demonstrate agonist action with B-S12-G7 | | [52] | D2-D3 – [52] |

**Table 1.** (Contd.)

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Common characteristic – inhibition of proliferation induced by any cytokine from IL-6 family (gp130 dimerization inhibition) | | | | |
| A1 | | XG-4-CNTF [45] | [29, 42, 45, 47] | D2 |
| A2/C33 | blocks proliferation induced by B1 + I2 | XG-2PA [47] | [29, 47] | D2 |
| A3 | agonist in pair with I1 (XG2) | XG1, XG2 [47] | [29, 39, 47, 51] | D2 |
| A4 | | | [29, 47] | D2 |
| I1/C41 | agonist in pair with C5, A3 or B2 (XG2) [39, 45], inhibits XG-4-CNTF proliferation in high concentrations [45] | XG-4-CNTF [45] | [29, 39, 45, 47, 51] | D3 |
| I2 | agonist in pair with B1 (XG2) | | [29, 39, 47, 51] | D3 |
| J1/C42 | | | [29, 47] | D3 |
| B-R3/C22 | strong effect, stronger than agonist B-S12 effect [52]; inhibits OSM signal in A375 cells (OSMR type II), impedes gp130 and LIFR chain association [45] | A375 [29], TF-1 [45] | [38, 40, 45, 52] | D2 – [38] |
| B-R12/C23 | | TF-1 [45] | [45] | D2 |
| B-S8/C24 | | TF-1 [45] | [45] | D2 |
| B-R23 | | TF-1 [45] | [45] | |
| B-A23/C18 | | | [40] | |
| GPX-7/C43 | | XG1, XG2, TF-1 | | D2 |
| GPX-22 | | XG1, XG2, TF-1 | | |
| Common characteristic – inhibition of proliferation induced by any cytokine from IL-6 family | | | | |
| D1 | inhibits proliferation in high concentrations | XG-4-CNTF | [29] | D4 |
| D2 | | XG1, XG2 | [29, 42] | D4 |
| D6 | | | | |
| B-T6/C15 | no functional effect | TF-1 | [45] | D4 |
| Function not known | | | | |
| D3 | | | [29] | D4 |
| D4/C36 | | | [29] | D4 |
| D5 | | | [29] | D4 |
| G1/C39 | | | [29, 47] | D1 |
| G2 | | XG-4-CNTF [29] | [29, 47] | |
| G3 | | | [29, 47] | |
| G4 | | | [29, 47] | D1 |
| H1/C40 | | | [29, 47] | D1 |

**Table 2.** Properties of cell lines used in studies of the mechanism of action of the extracellular part of gp130

| Cell line name | Origin, description | Types of receptors participating in gp130-mediated transduction | Reference |
|---|---|---|---|
| XG1 | human myeloma (plasma cell leukemia), IL-6-dependent | gp130, IL-6Rα | [63] |
| XG2 | human myeloma (pleural effusion), IL-6-dependent | gp130, IL-6Rα | [63] |
| XG-4 | human myeloma (plasma cell leukemia), IL-6-dependent | gp130, IL-6Rα, LIFR, CNTFRα | [67, 68] |
| XG-4-CNTF | human myeloma, subclone XG-4 proliferating in presence of CNTF | gp130, IL-6Rα, LIFR, CNTFRα | [67, 68] |
| XG-6 | human myeloma (plasma cell leukemia) | gp130, IL-11Rα | [67, 68] |
| XG-6-IL-11 | human myeloma, subclone XG-6 proliferating in presence of IL-11 | gp130, IL-11Rα | [67, 68] |
| XG-2PA | human myeloma, subclone XG-2 proliferating in presence of B1 + I2 mAbs | gp130, IL-6Rα (?) | [39] |
| TF-1 | erythroleukemia, IL-6-dependent | gp130, IL-6Rα (low conc.), (LIF, CNTFRα)? | [38, 45, 53] |
| SK-N-MC | human neuroblastoma | gp130, IL-6Rα, LIFR, CNTFRα, OSMR I | [45] |
| A375 | human melanoma | gp130, OSMR II, (IL-6Rα, LIFR, CNTFRα)? | [44, 45] |
| NIH3T3 | mouse fibroblasts | gp130, OSMR II, (IL-6Rα, LIF, CNTFRα)? | [45] |
| HepG2 | human hepatoma | gp130, IL-6Rα, OSMR I, OSMR II, (LIF, CNTFRα)? | [38, 40, 45, 52, 53] |
| Hep3B | human hepatoma | gp130, IL-11Rα | [44] |
| RPMI 8226 | myeloma, IL-6-independent | gp130, IL-6Rα | [53] |
| 2fTGH | fibrosarcoma | gp130 | [38] |
| BAF130 | mouse pre-B cells | gp130 | [53] |
| BAF80/130 | mouse pre-B cells transfected with IL-6R and human gp130 cDNA, IL-6-dependent | gp130, IL-6Rα | [39, 51] |
| BAF190/130 | mouse pre-B cells transfected with LIFR and human gp130 cDNA, LIF-dependent | gp130, LIFR | [39] |
| KG-1a | stem cell leukemia | gp130, IL-6Rα | [53] |

tion of cells in the presence of cytokine, and the cells lose this ability as the surface carrying the epitope specific for them disappears. This effect is clearly reproduced in flow cytometry experiments for different types of cells. Similarly [39, 47, 48], the behavior of other gp130 epitopes was studied (Fig. 4). In accordance with the fact that IL-6-specific blocking of group B antibody signal transmission was observed after IL-6 treatment, an assumption was proposed and confirmed about direct involvement of epitopes for mAbs of group B in the binding of preformed IL-6/IL-6Rα complex. In the presence of IL-6/IL-6Rα epitope for B becomes blocked by α-receptor gp80 and does not bind the corresponding mAb. In addition, the total number of activated and inactivated

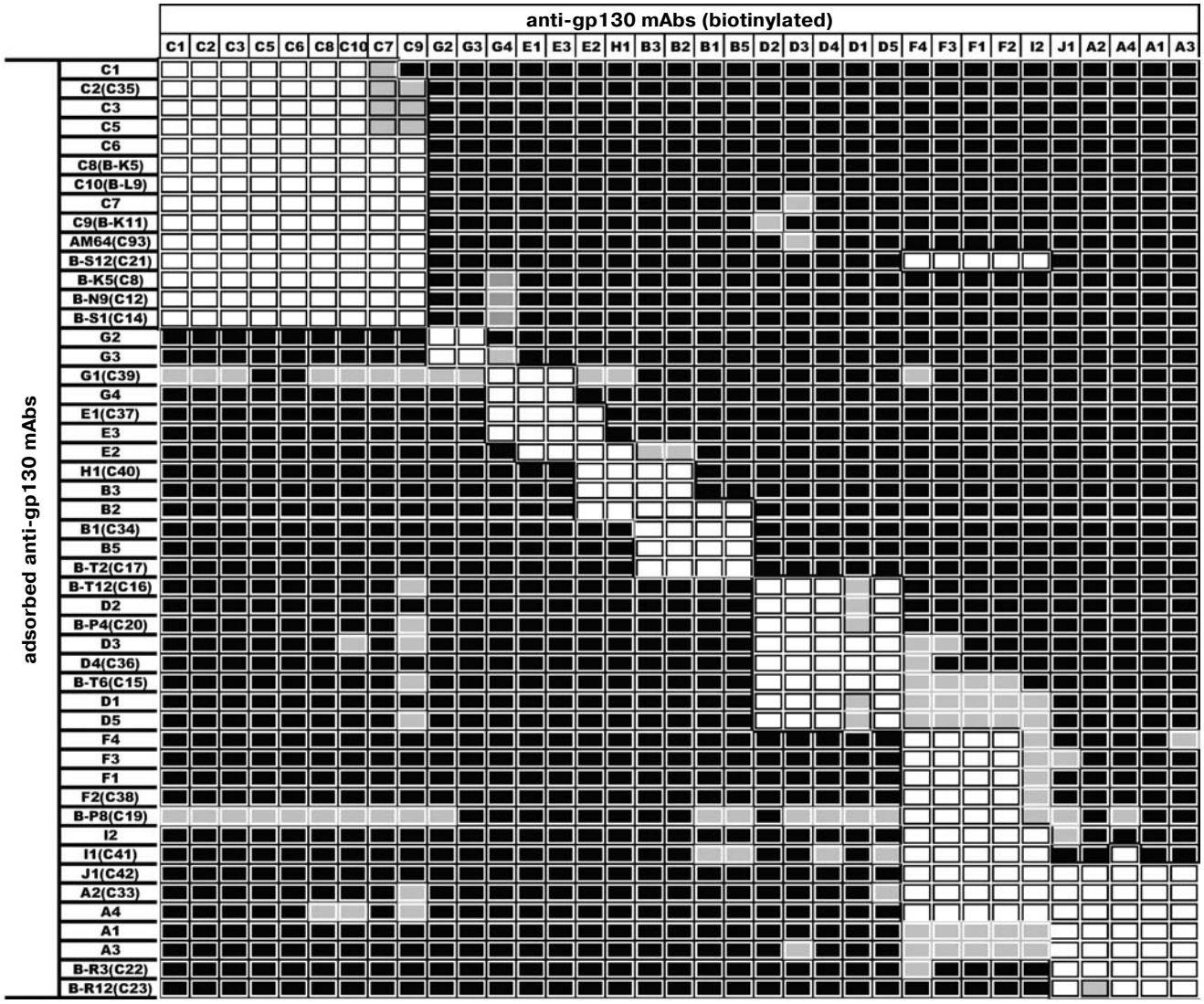


**Fig. 3.** Epitope mapping of gp130 extracellular part using anti-gp130 mAbs in accordance with [29, 38] and the VI International Workshop on Human Leukocyte Differentiation Antigens [40, 41]. White color indicates pairs of antibodies that recognize the same gp130 epitopes; gray color indicates substantially overlapping epitopes of mAbs; black color indicates mAbs pairs with different epitopes.

gp130 molecules on the cell surface of IL-6-sensitive lines can be estimated using mAbs C2, C7, D2, G4, H1 (their epitopes are not involved in IL-6 signal transduction); mAbs B1 and B2 reveal specific IL-6-mediated activation of the receptor, and mAbs of groups A and I serve as markers of gp130 homo- and heterodimerization and direct transmission of cytokine signals in the cell [39, 46, 48]. Thus, in this series of studies not only the structure and the functional role of the receptor was studied, but distinct immunophenotypes corresponding to gp130 activation states were also revealed.

**Application of gp130 immunophenotyping.** The described approach was used as a basis for clinical analysis of haematopoietic tumors, including B-cell non-Hodgkin's lymphoma [42, 43, 47]. Interestingly, the patients' cell immunophenotypes were more diverse than IL-6-induced activation states described after model cell lines in laboratory experiments. Further study of gp130 functional states formed in response to other cytokines from the gp130 family, such as LIF or OSM, may shed light on their role in the pathogenesis of cancer and many other diseases.

The estimation of recombinant human IL-6 functional activity based on monitoring of the gp130 activation states has found application in development of a high productivity method of cytokine expression in a bacterial system [49]. The gp130 immunophenotyping method is used for rapid evaluation of aptamers effectiveness on the cellular level in a project to develop a method of anti-cytokine therapy using RNA aptamers [50].

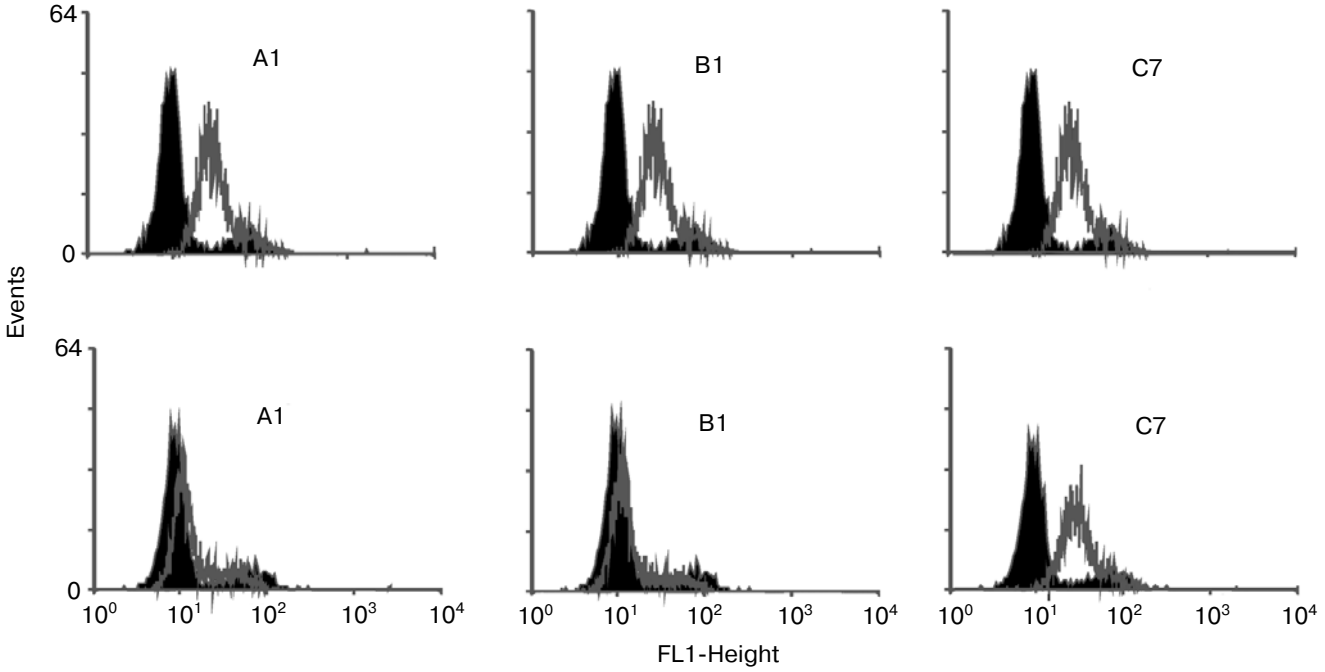


**Fig. 4.** Epitope structure changes (disappearance of epitopes for mAbs A and B) of gp130 under IL-6 treatment. RPMI 8226 cells were incubated with anti-gp130 mAbs A1, B1, or C7 and then stained with FITC-labeled $F(ab')_2$-fragments of anti-mouse antibody. Shaded peaks, non-specific cell fluorescence (isotypic control). Black line, fluorescence signal intensity of antibodies corresponding to different gp130 epitopes. Top row, peak positions corresponding to the binding of antibodies in the absence of IL-6 (non-activated receptor). Bottom row, peak positions corresponding to the binding of antibodies after cells preincubation (30 min, 4°C) in the presence of 100 ng/ml IL-6 (activated receptor).

## ACTIVATION OF gp130 WITH MONOCLONAL ANTIBODIES

Along with the ability of mAbs to block the transmission of signals of one or more cytokines, activating effect of certain antibodies was also detected, i.e. mAbs acted as gp130 agonists and induced cell proliferation in IL-6-dependent lines [38, 40, 41]. A closer examination of this phenomenon demonstrated that pairs of mAbs act as non-cytokine gp130 agonists [39, 51, 52], and such properties are observed for particular combinations of mAbs (Table 3) – a phenomenon that is clearly not explained by functional and steric proximity of specific epitopes [51, 53]. The existence of agonistic antibodies has been shown previously [54], so the discovered pairs became a powerful tool in subsequent detailed studies of the native gp130 activation mechanisms. The main conclusions of functional experiments on the replacement of IL-6 treatment of various cell cultures with mAbs stimulation are considered to be the following: i) confirmation of the existence of gp130 pre-activation states. The clearness of corresponding immunophenotype manifestation and transition rate between them is lower in the case of activation by antibodies; ii) detection of the need for two different regions of gp130 for its participation in signal transmission inside the cell; iii) structural mimicry as the primary mechanism of gp130 activation by antibodies; iv) discovery of a new method of differentiation stimulation in haematopoietic stem cells, and v) the possibility of development of clinical applications in haematology.

Because a large amount of experimental and factual material has been obtained by research groups in recent years, it has become necessary to organize this information and to discuss the most controversial issues and objectives for practical solutions. This paper also presents a comparison of experimental and theoretical data on the functional properties of non-cytokine activation and the clinical role of gp130.

## FUNCTIONAL ROLE OF EXTRACELLULAR DOMAINS OF gp130

In the light of a new data on the mechanisms and sites of cytokine binding with gp130, it is necessary to once again analyze the experimental data obtained for the anti-gp130 mAbs.

### 1. Domain Mapping of Anti-gp130 mAbs Epitopes

Several independent studies on the role of gp130 structural domains in signal transmission and binding to different cytokines have been conducted [34, 38, 52].

**Table 3.** mAbs that activate gp130

| mAb 1 | mAb 2 | Type of cells sensitive to mAb activation |
|---|---|---|
| B1 | I2 | XG1, XG2, BAF80/130, BAF130, XG-2PA, RPMI 8226 |
| B2 | I1 | XG1, XG2, BAF80/130 |
| B1 | F1 | XG1, XG2, RPMI 8226 |
| B2 | C5 | XG2 |
| A3 | I1 | XG1, XG2 |
| C5 | I1 | XG1, XG2 |
| B1 | anti-mouse IgG | XG2 |
| B2 | anti-mouse IgG | XG2, BAF80/130 |
| E1 | anti-mouse IgG | XG2 |
| B-S12-G7 | B-S12-A5 | RPMI 8226, HepG2, BAF130 |
| B-S12-G7 | Fab (B-S12-A5) | HepG2, BAF130 |
| B-S12-G7 | B-P8 | HepG2, BAF130 |
| B-S12-G7 | Fab (B-P8) | HepG2, BAF130 |
| B-S12 | B-P8 | HepG2, 2fTGH (synergism) |

**Domain D1 (IgD, N-terminal immunoglobulin-like membrane-distal domain of gp130).** B-P4 and B-P8 mAbs as well as subclones of agonistic antibody B-S12 were shown to not affect the D1 domain of gp130 [52], while the mapping of the B-T2 mAbs revealed the presence of its epitope in this domain [34]. The necessity of D1 for IL-6-, IL-11-, and CNTF-induced activation of gp130 was clearly proved [32, 34]. D1 is not involved in LIF-signaling [32]; comparison of IgD domain structures of gp130 and LIFR suggests [15] that OSM is able to bind D1 in the case of low LIFR concentration. These data and conclusions [15, 29], made on the basis of mAbs epitope mapping, suggest that the D1 domain can contain interaction epitopes for mAbs that inhibit IL-6-, IL-11-, and CNTF-, but not LIF- or OSM-induced cellular response.

**Domain D2 (part of cytokine-binding homology region, CHR).** Domain mapping of D2 domain has been performed only for B-R3, B-P8, and B-S12 antibodies and confirmed for the B-R3 [38]. However, this domain can bind to any functionally active mAb.

**Domain D3 (part of cytokine-binding homology region, CHR).** Binding to this domain was investigated with B-R3, B-P8, B-P4, B-S12, and individual subclones of B-S12 (B-S12-A5 and B-S12-G7) and proved for B-P8 and B-S12 [38]; the structure of gp130 deletion mutants only allowed indicating epitope belonging of B-S12-A5 and B-S12-G7 to the D2-D3 site [52]. The role of D3 as well as D2 in the binding of cytokines is very important and may manifest itself in the form of functional activity of all antibodies.

In addition, the D2 and D3 domains contain sites not involved in the binding of ligands; the functional role of mAbs against such sites may be various.

**Domains D4, D5, and D6 (fibronectin type-III domains forming long membrane-proximal stalk of receptor).** Among B-R3, B-P8, B-S12, and B-P4 [52], only B-P4 binds to this part of the receptor (domain D4). D4-D6 domains were demonstrated by construction of chimeric proteins [55] to affect the binding of ligands and gp130 signal transduction. There are suggestions of direct involvement of D5 in the heterodimerization [56] or of D6 in the homodimerization [54] of gp130. A special role is noted for D5 [58], whose replacement by homologous domain from granulocyte colony-stimulating factor receptor (G-CSFR) does not prevent the binding of cytokines and leads to ligand-independent activation of the receptor. mAbs binding to these domains may non-specifically affect the ability of gp130 to bind cytokines and block signal transmission as well as be selective epitopes for IL-6 and IL-11 as supposed in [59] because of the high steric density of hexameric complexes they form.

## 2. Specificity of Inhibition by Monoclonal Antibodies of the Effect of Particular Cytokines

A vast area of overlapping cytokine binding surfaces in CHR-domain [13], thermodynamic plasticity of gp130 IgD-domain [13, 22], and structural homology between IgD of gp130 and site II of cytokines [15] indicate some common effects of cytokines on gp130 recognizing sites, which suggests the absence of strictly selective mAb action on the binding sites of specific cytokines. On the other hand, this type of action cannot be excluded because: i) each bound ligand has unique interactions with gp130, located in the peripheral area of the CHR domain; ii) the role of the IgD domain is clearly described for the formation of specific receptor complexes; iii) membrane-proximal domains (D4-D6) can selectively restrict the mobility of receptor chains, making transmission of the signal even more dependent on the properties of the ligand.

Careful examination of all available data on the effect of mAbs on the cytokine-mediated proliferation (with consideration of cell types and receptors expressed on their membranes, Table 2) and the epitope mapping results (Fig. 3) presented in [29, 38, 40, 45] suggests the epitopes domain location for different groups of mAbs. The IgD domain exposes epitopes detected by antibodies of groups B and E because it is specifically involved in IL-

6 signaling, and B-T2 from this group, typical in specificity, binds to IgD [34]. Similar logic leads to the conclusion about the epitope locations of mAbs of groups A, B-R3, A3, A4, and B-R12 in D2 domain; mAbs of groups I, J, F, B-P8 in D2 or D3 domain; mAbs C1-C10, B-K5, B-S1, B-N9, AM64 in D3, presumably in the region not belonging to CHR, but interacting with LIFR/LIF or LIFR/OSM complexes. Epitopes of mAbs of group D as well as B-T6, B-T12 may be located in membrane-proximal domain D4, as well as B-P4, their neighbor in the epitope mapping. To confirm these hypotheses, pre-activation immunophenotypes of cells with mAb-blocked gp130 domains can be described. Such experiments are finer counterparts for the domain mapping experiments with mutant or chimeric proteins.

### 3. Activation of gp130 with mAbs

The ability of certain antibodies to bring cytokine receptor into the activated state shows the common properties of ligand-binding receptor sites and helps to formulate the paradigms of reception and transduction for the entire pool of recognizable molecules.

Identified agonist pairs of anti-gp130 mAbs act, as assumed in [52], according to the mechanism of structural mimicry and/or as a result of the conformational modification of certain receptor sites. Also in favor of these assumptions, there are observations [37, 59, 60] that a certain orientation of the receptor chains caused by non-specific ligand, or even a modified antagonist, could lead to the activation and normal functioning of the receptor.

Taking into account the need to modify two different sites for gp130 signal transduction [14, 33, 34], participation of exactly two mAbs in the formation of activated gp130 complex seems logical; it should be noted that each of the antibodies in a pair corresponds to a different part of gp130 according to the data of epitope mapping (Table 3), except perhaps the A3 + I1 pair.

If we adhere to the above assumption on the assignment of antibody domains, all activating pairs of mAbs can be divided into groups: D1 + D2, D1 + D3, D2 + D3 (the last probably includes pair A3 + I1, since in spite of common functional effect of antibodies A3 and I1, their epitopes are rather substantially spatially separated, Fig. 3). In addition, combination of mAbs with domains D2 + D3 probably simulates the system of gp130 activation with LIFR-containing complex. Interestingly, the combination of D1 + D3 corresponds to only one pair of antibodies, B2 + C5; on one hand, this speaks in favor of the steric mechanism of receptor activation, on the other hand, it indicates the key role of D2 domain in gp130 activation by any ligand. To test the reviewed hypothesis of signal transmission, one can use immunophenotyping of gp130 activation states or tests on cell proliferation in the presence of mAbs, one of which will block one of the sites that is essential for signal transmission, and the pair of the other two mAbs will restore activity. Comprehensive staging of experiment series may detect the activation of heterodimerized chains of LIFR and gp130 in the presence of anti-gp130 mAbs.

Simple one-dimensional flow cytometry histograms of mAbs binding also indicate rather a steric, noncovalent dimerization process of the receptor in the presence of agonists antibodies [53]: the peaks of characteristic antibody A1, which describes the state of gp130 dimerization site, not only shift to the control region, which corresponds to reduction in the number of unactivated gp130 chains, but also become more obtuse, demonstrating the presence of A1 gradient that can be attributed to the partial exposure of A1 epitopes, which was caused by incomplete convergence of chains in the process of non-cytokine activation.

Another controversial question about the mechanism of mAb agonistic action arises when considering their active concentrations. It was noted [51] that although required concentrations of mAb agonists are relatively high, in fact only the presence of one mAb from a pair in this concentration is needed, whereas the concentration of the second mAb may be 10 times smaller. Given that gp130 is a sensitive receptor, effectively transmitting signals *in vitro* in the range of tens of pg/ml (in the case of IL-6) [29], acting concentration of mAbs (μg/ml) are high. It is possible that the binding of the first antibody to gp130 is the rate-limiting stage, and the addition of the second mAb rapidly conducts the signal from preformed dimer [61] into the cell; the active complex itself may be conformationally not optimal in the process. Maybe this is the reason for retarded initial stage of cell proliferation noted in [39] and later phosphorylation of intracellular proteins in response to non-cytokine activation of gp130.

### 4. Conservatism of Structure of gp130 Extracellular Domains

One of the most important properties of transducer receptor gp130 is its pleiotropy not only among ligands of one organism, but also with respect to conservative action in mammals. There are interspecies differences in cytokine structure and architecture of receptor complexes, for example, between human and mouse. The mIL-6 and mIL-6Rα from mice do not interact with human counterparts [62], and mOSM does not bind mLIFR [63]. However, gp130 is able to mediate signal transduction for ligands of different kinds. Evolutionary significance of this feature is an indicator of a sort of high organization of biochemical forethought of cellular mechanisms of regulation. The principle of structural rigidity, proposed by C. Garcia with coauthor [13], or "thermodynamic plasticity" of gp130 cytokine-binding sites may serve as a structural explanation for this phe-

nomenon; it allows the receptor to bind various ligands, including their synthetic agonists and antagonists. The most significant example of such interaction to date is the activation of gp130 by homolog of human interleukin-6 – viral IL-6 (vIL-6). It is encoded in the genome of human herpes virus type 8 and is associated with Kaposi's sarcoma, body cavities lymphomas, and multicentric plasma cell variant Castleman's disease [64]. Despite high homology between viral and human IL-6, the mechanisms of gp130 activation by them are fundamentally different: vIL-6 does not make preformed complex with IL-6Rα and induces cytokine signal not in hexamer but in tetramer complex with gp130. In this case, it is much less tissue-specific and induces haematopoiesis, plasmacytosis, and angiogenesis.

### 5. Clinical Monitoring of gp130 Activation Level and Therapeutic Approaches for Hemoblastoses

High gp130 activation level in multiple myeloma and non-Hodgkin's lymphoma indicates the necessity of choosing a target for anti-IL-6 therapy: the etiopathogenetic component may be represented by viral or human IL-6 [65, 66]. Differences in the molecule topology of these cytokines allow specific attack of their unique antigenic determinants with mAb. Future clinical applications, associated primarily with regulation of haematopoiesis and hemoblastoses therapy, can be developed through the use of knowledge about the mechanisms of cytokine signal transmission and features of gp130 structure.

Current data on the structure, functions, and action mechanisms of gp130 receptor obtained by several independent research groups enable the development of methods to influence the fine subdomain structure of the molecule and to cause a variety of functional cell responses: from acute phase response and to specific differentiation signals. Development of cancer and autoimmune disease therapies using monoclonal antibodies to certain parts of gp130 or to the corresponding sites of receptor–cytokine complexes components is urgent. New signaling gp130-activating molecules were recently discovered, including viruses. An important place in the studies of gp130 is occupied by descriptions of heterodimeric receptor complex architectures of known cytokines from IL-6 family. Detailed knowledge of gp130 structural features opens up prospects for the development of highly specific cytokine antagonists and methods of non-cytokine receptor activation.

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