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Cytokine receptor interactions as drug targets

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Abstract

Cytokines are essential proteins that exert potent control over entire cell populations to fight infections and other pathologies, but can by themselves cause disease. Therefore, cytokine related drugs act either by stimulating or blocking their activities. Our knowledge of the structures of cytokine-receptor complexes, the biophysical basis of their binding, and their mode of biological activation has substantially increased in recent years. This knowledge has been translated into new drugs and drug candidates. This review summarizes our current understanding of the receptor-mediated activity of cytokines, their relation to health and disease, and the agents in use to activate and block their actions.

Cytokines, such as interleukins (IL-) and interferons (IFN), are secreted proteins that carry intra- and inter-cellular signals that regulate the immune response to pathogens or other pathological conditions. For their function, cytokines must bind to specific extracellular receptors. Some cytokines, such as the type I interferons, have dedicated receptor chains (IFNAR1 and 2). However, other cytokines bind to both dedicated and shared receptors [1]. Two examples of cytokines that bind shared receptors are IL-4 and IL-13, which bind combinations of IL-4R α , γ_c and IL-13R α [2*], and the different modes of interaction of GP130/CNTF-R α /LIF-R with CNTF versus GP130/IL-6R α with IL-6 [3*]. A combination of structural biology and biophysical methods has provided detailed information on many cytokine-receptor complexes. These studies have revealed that one receptor chain often functions as the “binding” receptor, exhibiting nM affinity, while the other receptor functions as the “signaling” receptor making only weak (μ M affinity) interactions within the cytokine receptor complex.

The mechanism of cytokine-mediated receptor activation remains controversial. One idea is that cytokine receptors are pre-assembled, or pre-concentrated on the cell surface, and activation occurs by structural changes in the receptors upon ligand binding. A competing model suggests that cytokine receptors are dispersed within a region of the membrane and cytokine binding “oligomerizes” the receptors. A major issue is the role of structural rearrangement versus receptor aggregation upon cytokine binding. FRET and other experiments have shown pre-assembly of the receptor chains for IL-5 [4*], IFN γ R1 [5], GP130 [6], IL-10R1 [7] and others. In addition to pre-assembly of receptor chains, it was also suggested that some of the receptors are clustered on the membrane, facilitating rapid complex formation and cellular signaling. A good example for this concept is provided by

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studies on the IL-2 and IL-15 receptors [8,9]. If indeed receptors are at least partially pre-localized, transmission of the signal should involve structural rearrangements to prevent random signaling. There is mounting evidence that, at least in some cases, receptor assembly requires a “structural signal” that is transmitted through the membrane. This model has been suggested for the IL-4 [10], human growth hormone [11], erythropoietin [12] and type I interferon signaling complexes [13]. However, this data does not exclude the possibility that some cytokines relay their signal by simply driving receptor oligomerization.

While most drugs are small molecules, cytokine related drugs are mostly proteins. Producing small molecule agonists for cytokines is extremely difficult due to the large distance between the receptor chains that is bridged by the cytokine. An exception is the EPO mimicking peptide (Hematide, Affymax) that is now in phase 3 clinical trials. Production of small molecule antagonists, that block the cytokine-receptor interaction, may be more feasible, as stable binding to only one of the proteins has to be achieved. Still, competing any protein-protein interaction with a small molecule is a difficult task [14]. Indeed, no approved small-molecule drug has yet been produced for this important target class. Because of the tremendous importance of cytokines in health and disease, extensively efforts are being made to identify potent antagonists and agonists of cytokines. Molecules identified to date have been used to understand the mode of action of cytokines and as therapies to treat a variety of conditions including immune disorders, cancer, and infectious diseases. Below we describe examples of the quest to find better agonists and antagonists of cytokines (for a summary see Table 1 and Figure 1).

Type I interferons

Among the better-characterized cytokines are Type I interferons, comprising 13 α subtypes, one β , κ , ω and ε subtype, which all bind a receptor complex consisting of IFNAR1 and IFNAR2. All alpha interferons, except IFN α 1, bind IFNAR2 with nM affinity and IFNAR1 with $\sim\mu$ M affinity [15–17]. The binding properties of two IFN's stand out, α 1 is the weakest binding IFN (20-fold lower than other IFN α subtypes), and IFN β forms the tightest interaction with the IFNARs ($\sim$ 500 fold tighter than other subtypes) [16,17]. Careful studies on artificial membranes have shown that mimicking the assembly of the ternary interferon-receptor complex at physiological interferon and receptor concentrations results in a ternary complex with a half-life of minutes [16]. However, at higher receptor or the ligand concentrations or when applying IFN β (which binds tighter) the half-life of the receptor complex extends to hours [13,16,18*]. Not surprisingly, IFN β has been connected with the ability to induce unique cellular activities in addition to the common antiviral effects shared by all type I interferons. These include increased antiproliferative activity [19], decreased tumorigenic cell growth [20], differential regulation of cell cycle [21], prolonged receptor down-regulation [18*,22], specific gene induction [18*,23] and more.

Interferons were early on recognized for their medical use, initially as antiviral drugs and later for the treatment of some forms of cancers as well as being the most common treatment against multiple sclerosis [24], leading to a quest to find better IFN agonists and antagonists. While a high-resolution structure of the ternary interferon-receptor complex remains elusive, a combination of extensive mutagenesis and NMR studies have enabled reasonable modeling of this complex [18*,25–27]. These studies revealed that the two receptors bind on opposite, independent surfaces on interferon, and suggest that all type I interferons use the same binding sites on the receptors. The apparently differential activities promoted by the different interferons (particularly α versus β), were found to relate to the binding affinity towards the two-receptor subunits, the concentration of interferon used, the density of cell surface receptors, and the time of activation [18*,28–30]. It was shown that by increasing the binding affinity of alpha-interferon (through protein engineering) to that measured for

IFN β , one can mimic the biological activity of the latter, as well as the IFN β -specific gene activation pattern [18*,28,29]. Alanine scanning mutagenesis of IFN α 2 has revealed three conserved positions (57, 58 and 61 in all human IFN's) on the IFNAR1 binding surface that when mutated to Ala resulted in an increased binding affinity [31]. This suggests that weak binding to IFNAR1 is required for optimal activity of alpha-interferons. It should be noted that mutations to Ala increased binding also between IL-2 and IL-2R α [32], suggesting that non-optimal receptor binding is a common trait for cytokines. Further binding optimization, using phage display of positions 57, 58 and 61, resulted in a strong interferon agonist (termed YNS). The triple mutant binds IFNAR1 20–100 fold more tightly than any other IFN α and 3 fold more tightly than IFN β . Binding of YNS to IFNAR2 was comparable to wild-type IFN α 2. The YNS mutant conferred ~100-fold higher antiproliferative potency on WISH and MDA231 cells compared to IFN α 2, while its antiviral activity was increased by only 2-fold. Further increase in binding affinity did not result in higher activity (GS unpublished) suggesting that while maximum antiviral activity is reached already by IFN α 2, the YNS mutant reaches maximal antiproliferative potency. The increased potency of YNS resulted in complete elimination of cancer in a xenograft model [29]. Compensating mutations on the IFNAR2 binding site demonstrated that the increased antiproliferative activity is a result of the stabilization of the ternary complex, and not due to tighter binding specifically to either one of the receptors [18*]. Other interferon agonists have been developed, including a 13 mer peptide [33], a consensus alpha-interferon [34], and an IFN α 8 mutant optimized to bind tighter to IFNAR2 [35]. However, none of them reaches the activity level of either IFN β or the YNS mutant. Currently, IFN α 's are used to treat cancer and viral infections, while IFN β is used to treat Multiple Sclerosis. The success of IFN treatment is due to its multiple functions, which include antiviral, antiproliferative and immunomodulatory activities (IFN can activate both pro and anti-inflammatory responses [36]). Our improved understanding of differential type I interferon activities suggests that the historical choices of interferons used in the clinic should be revisited.

As found for other cytokines, a dysfunctional interferon system can lead to the development of different disease conditions. Deficiency in functional interferon results in increased susceptibility to viral infections as well as tumor growth [37]. There is also mounting evidence that Type I interferons are involved in systemic lupus erythematosus (SLE) development and/or progression [38,39]. Therefore, efforts have been invested in producing potent interferon antagonists. These can be divided into four groups, those that are based on interferon, the IFN receptor, small molecules, and antibodies (see Figure 1 and Table 1). An anti-IFN α monoclonal antibody (MEDI-545) was developed to eliminate IFN from the system and is now in phase I clinical trial against SLE [40]. Similarly, soluble IFNAR2 was evaluated for its antagonist or agonist activity in mice. Interestingly, even when using a 50-fold tighter binding IFNAR2 mutant, the receptor acted as a soluble binding protein, enhancing the half-life of interferon in the system (but reducing its free concentration) [41]. Theoretical simulations have shown this to be a result of the very low IFN concentration (0.3 pM) needed to confer antiviral activity, undermining this approach [41]. Viruses have evolved many strategies to avoid the antiviral effects of interferons [42]. An IFN evasion strategy, particular to poxviruses, is the expression of a secreted IFN decoy receptor, IFN α / β BP [43,44]. This receptor binds interferon at high affinity (subnanomolar) and blocks the antiviral effect of interferons [45]. It should be noted that IFN α / β BP is also active when bound to the membrane, and thus its mode of inhibition is different from that of a soluble receptor.

A different strategy to hinder IFN activity is by blocking the receptors. Small molecule non-peptidic IFN receptor binders were synthesized by mimicking the structural features of receptor recognition. Two compounds that exhibited antagonist activity (blocking IFN-inducible Stat phosphorylation) were found, however with relatively weak binding affinity

[46]. Antibodies raised against the IFNAR receptors were also shown to efficiently hinder IFN action [47]. Another strategy to produce an effective antagonist was by nullifying the IFNAR1 binding site on interferon (by mutating residues 65, 80, 85, 89 and 120) and enhancing IFNAR2 binding (by replacing the C-terminal tail of IFN α 2 with that of α 8) [26*, 31,48]. This engineered IFN variant was shown to completely block wild-type interferon activity even at high agonist concentrations [26*]. When applying an efficient IFN antagonist one should remember that systemic IFN blockade may cause increased viral susceptibility [24], which will require tight monitoring to permit the success of this strategy.

Type II Interferons

In addition to the type I IFNs, cells produce a single Type II IFN molecule, called IFN γ . As implied by its name, IFN γ also exhibits antiviral activities, but it is less potent than IFN α/β . The main function of IFN γ is to activate macrophages and natural killer cells against intracellular pathogens, which results in direct cell killing and shaping of the adaptive immune response. IFN γ binds to a distinct receptor comprising the IFN γ R1 and IFN γ R2 chains, which function as the high and low affinity binding chains, respectively. In contrast to IFN α/β , IFN γ forms a homodimer and additionally contains a flexible positively charged C-terminal tail. Two crystal structures of the IFN γ /IFN γ R1 complex reveal its overall dimeric structure [49] and the details of the cytokine-receptor interface [50]. Surprisingly, the C-terminal tail of IFN γ was not observed in either crystal structure, suggesting it is not important for IFN γ R1 binding. Subsequent binding studies [51] revealed the IFN γ C-terminal tail increased the on-rate of the IFN γ /IFN γ R1 interaction but not the stability of the complex. In addition, the C-terminal tail binds strongly to heparin and increases the serum half-life of the cytokine [52]. The *in vivo* function of the IFN γ C-terminal tail remains unclear, but these studies suggest that receptor independent mechanisms regulate the stability of the cytokine and possibly its targeting to certain cells or organs.

Like IFN α/β , IFN γ is antagonized during poxvirus infection, by the secreted poxvirus IFN γ binding protein (IFN γ BP). Structural characterization of the IFN γ /IFN γ BP complex has revealed novel insights into this virally designed IFN γ antagonist [53*]. The 268 amino acid long IFN γ BP can be broken down into an IFN γ R1-like domain (20% sequence identity with IFN γ R1) and a novel 58 amino acid tetramerization domain (D3). The function of the D3 domain is to assemble the IFN γ R1-like chains with precise geometry that mimics the dimeric IFN γ /IFN γ R1 cell surface complex. As a result, each IFN γ BP tetramer binds two IFN γ dimers. In contrast to the IFN γ /IFN γ R1 complex, the IFN γ C-terminal tail is tightly bound to IFN γ BP in the IFN γ /IFN γ BP complex, which further suggest an important role of IFN γ in the clearance of the virus. As a result of the extensive bivalent contacts, the IFN γ /IFN γ BP interaction is much tighter than the cellular IFN γ /IFN γ R1 complex. Thus, IFN γ BP is an optimized variant of the extracellular IFN γ R1 chain, which the virus designed with a novel IFN γ binding interface, quaternary structure, and geometry of the IFN γ binding chains.

A humanized anti-IFN γ monoclonal Ab has been prepared (HuZAF) and tested for safety in Crohn's disease [54]. The original Ab used for humanization was a mouse anti-IFN γ antibody (AF2) selected based on its ability to inhibit IFN γ mediated expression of MHC Class II molecules on a human melanoma cell line [55]. Epitope mapping revealed AF2 bound to an IFN γ epitope outside of the IFN γ /IFN γ R1 contact surface near IFN γ residues 65 and 103, which are located on the top of the V-shaped IFN γ dimer. The antibody was humanized, to create HuZAF, using sequence and structure-based methods [55,56].

Interleukins

The IL-1 family

The IL-1 family of cytokines includes IL-1 α , IL-1 β , IL-18, and IL-33 (for reviews, see [57,58]). IL-1 binding drives the assembly of a heterodimer of IL-1 receptor chains, a high-affinity specific IL-1R1 receptor and a low-affinity core-receptor IL-1RAcP, to produce a pro-inflammatory response [59] that can cause severe pathologic effects if not carefully regulated. A number of natural and engineered antagonists exist for this system, including IL-1 receptor antagonist (IL-1Ra), production of which is stimulated by many substances including IgG, cytokines, and bacterial or viral components. IL-1Ra competes with IL-1 α and IL-1 β for binding to IL-1R1 with which it forms a non-productive complex. A second natural antagonist of IL-1 is IL-1R2, which is a non-productive receptor (lacking the intracellular TIR domain) that competes for binding of IL-1 [60]. The use of neutralizing anti-IL-1Ra antibodies demonstrated that IL-1Ra is an important natural anti-inflammatory protein. Recently, an IL-1Ra mutant with enhanced antagonistic activity was developed using directed evolution, for potential therapeutic use. This engineered variant has nine-fold enhanced antagonistic activity relative to wild-type [61]. A recombinant form of IL-1Ra (Anakinra) is used in the treatment of rheumatoid arthritis, an autoimmune disease in which IL-1 plays a key role. Finally, IL-18, which binds two receptors, IL-18Ra and IL-18R β (which are very similar to IL-1R1 and IL-1RAcP) is occluded from the system by the natural antagonist IL-18 binding protein (IL-18bp) [57].

The IL-2 family

IL-2 is a member of a family of cytokines that include IL-4, IL-7, IL-9, IL-13, IL-15 and IL-21. They all share a common γ_c receptor chain in addition to a ligand-specific receptor chain (for review see [1]). IL-2 is necessary for the development of T cell immunologic memory. Many of the immunosuppressive drugs and drug candidates that treat autoimmunity inhibit IL-2 signaling. For example, SP4206 is a small molecule that binds IL-2 with an affinity of 70 nM and blocks binding to IL-2Ra [32]. SP4206 was assembled from smaller fragments. A detailed analysis of its mode of binding has shown it to target the same hotspot residues on IL-2 as does one component of the IL-2 receptor. However, the exact contacts are different, suggesting multiple possible solutions for high affinity binding [32]. Another IL-2 antagonist is the monoclonal antibody to IL-2Ra (Daclizumab), which is effective in preventing the rejection of organ transplants [62]. Other small-molecule inhibitors of IL-2Ra have also been reported [63]. IL-2 (Proleukin) itself is used to augment immune system function and has efficacy in treating metastatic renal carcinoma and melanoma.

IL-5

IL-5 is a member of a cytokine family that also includes IL-3 and GM-CSF. They all bind a common β_c receptor subunit in addition to their specific receptor. IL-5 exerts hematopoietic functions, and is produced by T helper2 cells and mast cells. Its functions are to stimulate B cell growth and increase immunoglobulin secretion. A cyclic peptide of 18 aa (AF17121) was selected to bind IL-5Ra with a binding affinity of 23 nM [64]. Epitope mapping has shown it to interact at an overlapping region to IL-5. In a cell assay, this peptide was shown to be a specific IL-5 antagonist with an EC₅₀ of 50 nM for blocking IL-5-dependent eosinophil activation. AF17121 also inhibited the accumulation of a FRET signal upon addition of IL-5 to CFP and YFP labeled receptors, suggesting that their dimerization drives activation [4*]. Interestingly, the same studies also suggest that the active receptor complex includes more than one copy of each receptor, with preformed β_c receptor oligomers enhancing the rate of cellular response.

IL-17

IL-17A is a pro-inflammatory cytokine produced by the Th17 subset of T-cells [65]. It mediates their effects through the IL-17 receptor family, of which there are five related members (IL-17RA IL-17RE). A human monoclonal antibody to IL-17A was shown to neutralize IL-17A activity by binding to the previously predicted receptor-binding cavity. The affinity of the Ab to IL-17A is 2.1 nM, resulting in an EC₅₀ of ~1 nM as measured in a cell assay [66*].

IL-22

IL-22 is part of the IFN γ gene locus, and is a member of the IL-10 family of cytokines that include IL-10, IL-19, IL-20, IL-24, and IL-26 [67]. IL-22 activates epithelial cells to induce anti-microbial peptides and protects the integrity of mucosal surfaces. IL-22 binds to a hetero-dimeric cell surface complex consisting of the high affinity IL-22R1 chain and the low affinity IL-10R2. In contrast to IFN α/β and IFN γ receptors, IL-22R1 and IL-10R2 are both shared receptor chains. IL-22R1 also pairs with the IL-20R2 chain leading to IL-20 and IL-24 signaling. IL-10R2 is required to activate IL-10, IL-22, IL-26, IL-28, and IL-29 activity [67]. Crystal structures of IL-22/IL-22R1 complexes [68,69*] and IL-10/IL-10R1 [70] reveal structurally similar binding modes for both receptor complexes. Interestingly, specificity is derived from the most structurally similar regions of the receptors and ligands. In particular, a conserved tyrosine residue on IL-22R1 and IL-10R1 functions as a hook which is inserted into a small cleft made by the AB loop of the cytokines [69*]. The contacts in this interaction dictate the overall orientation of the cytokines on the receptor surfaces, which generate differences in specificity and affinity. Thus, alterations in the AB loop can have a dramatic impact on receptor binding and function. Interestingly, the location of the epitope for the anti-IFN γ antibody HuZAF, mentioned above, is near the IFN γ AB loop, which suggests HuZAF-mediated IFN γ neutralization might occur at least partially through this mechanism. Furthermore, structural studies on an anti-IL-10Ab/IL-10 complex revealed deformation of the AB loop on IL-10 as a mechanism by which IL-10R1 binding was disrupted [71]. Because the AB loop on the cytokines forms a cleft, this region of the structure might be druggable with small molecules. In addition to the high affinity IL-22/IL-22R1 interaction, alanine scanning mutagenesis has revealed the IL-10R2 binding epitope, which exhibits an affinity of ~14 μ M [69*]. Mutation of IL-22 residues Y51 and N54 drastically disrupts IL-10R2 binding in vitro and IL-22 activity in cell based assays [72]. The structure of the IL-10R2 chain, and a data-driven computational docking model of the IL-22/IL-22R1/IL-10R2 ternary complex, provided additional insight into the IL-22/IL-10R2 interaction. Thus, as described previously for IFN α/β , an IL-22 molecule that retains the ability to bind IL-22R1 but cannot bind the IL-10R2 chain or induce subsequent activity has been made. To further develop the molecule as a suitable antagonist will require affinity maturation of the IL-22/IL-22R1 interface to increase its affinity.

Biological data suggested that IL-22 antagonism should be pursued as therapies for psoriasis and rheumatoid arthritis [73]. As a result, an anti-IL-22 Ab has been developed by Wyeth-Pfizer (Patent WO 2007/098170). Initial studies identified two rat antibodies that bound to IL-22 at positions overlapping the IL-22R1 (Ab04) and IL-10R2 (Ab02) binding sites [74]. However, the highest affinity and most potent neutralizing anti-IL-22 antibody (356A11) was obtained using phage display technology. Ab 356A11 exhibits an IC₅₀ of 53 pM in a BAF3 cell line expressing IL-22R1, and a dissociation constant for the 356A11/IL-22 interaction of 54 pM. The 356A11 Ab recognizes both human and mouse IL-22 with high affinity, allowing preclinical studies in animal models.

Cells naturally encode a soluble IL-22 binding protein (IL-22BP), which binds to IL-22 with pM affinity [69*]. IL-22BP is an extremely potent IL-22 antagonist. The biology of IL-22BP

has not been thoroughly investigated. However, the presence of IL-22BP in the genome suggests that cells at some point require the ability to efficiently block IL-22 activity. IL-22BP shares ~20% sequence identity with IL-22R1. As expected by the sequence similarity to IL-22R1, the crystal structure of the IL-22/IL-22BP complex [75*] reveals the IL-22/IL-22R1 and IL-22/IL-22BP interfaces are similar, except that the IL-22/IL-22BP interface is larger.

Conclusions

Due to their important role in health and disease, controlling cytokine action remains a major focus of drug discovery efforts. Efforts to date have led to multiple approved drugs with estimated annual revenues of >20 billion dollars. Many cytokines are themselves drugs, used with varying degrees of success to treat viral or bacterial infections (IFN- α , IFN- γ), multiple sclerosis (IFN β), and various forms of cancer (IFN- α , GM-CSF, and IL-2). Because cytokines activate inflammation, they contribute to chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and Crohn's disease. This has led to the development of cytokine antagonists, some of which have been described in this review. Cytokine-receptor complexes are protein-protein interactions, which are difficult to block using small molecules. Thus, at this time, humanized antibodies are the preferred strategy as cytokine antagonists (Table 1). While this seems quite straightforward, the multiple activities of many cytokines (pleiotropy), can lead to unexpected outcomes in patients. Thus, a better understanding of receptor-mediated cytokine activation is needed to enable the design of improved cytokine agonists and antagonists. Ongoing studies of "virally designed" cytokine antagonists (e.g. IFN α/β BP and IFN γ BP) provide one method to further such optimization. The use of library-based protein design, combined with novel selection strategies based on functional criteria rather than binding, holds great promise for improved cytokine modulators to further improve human health. Small molecule drug design for cytokine receptors, at this time, remains elusive.

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References and Recommended reading

1. Wang X, Lupardus P, Laporte SL, Garcia KC. Structural biology of shared cytokine receptors. *Annu Rev Immunol* 2009;27:29–60. [PubMed: 18817510]
- 2*. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, Keegan AD, Garcia KC. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* 2008;132:259–272. In this manuscript the authors describe the mechanism of pleiotropic signaling and suggest that the biophysics of cytokine receptor interaction on the outside of the cell can influence divergent signaling responses through shared receptors. [PubMed: 18243101]
- 3*. Skiniotis G, Lupardus PJ, Martick M, Walz T, Garcia KC. Structural organization of a full-length gp130/LIF-R cytokine receptor transmembrane complex. *Mol Cell* 2008;31:737–748. Here the authors show how the same receptor molecule (gp130) can adopt different conformations when binding to different ligands and co-receptors and how this can translate to a different modes of cellular activation. [PubMed: 18775332]
- 4*. Zaks-Zilberman M, Harrington AE, Ishino T, Chaiken IM. Interleukin-5 receptor subunit oligomerization and rearrangement revealed by fluorescence resonance energy transfer imaging. *J Biol Chem* 2008;283:13398–13406. Using FRET to follow the assembly of the IL-5 receptor composed of βc and IL-5R α upon ligand binding showed that βc is pre-aggregated on the cell membrane, enhancing the rate of cellular response. Contrary, IL-5R α binds (and forms the signaling complex) only upon the addition of IL-5.

5. Krause CD, Lavnikova N, Xie J, Mei E, Mirochnitchenko OV, Jia Y, Hochstrasser RM, Pestka S. Preassembly and ligand-induced restructuring of the chains of the IFN-gamma receptor complex: the roles of Jak kinases, Stat1 and the receptor chains. *Cell Res* 2006;16:55–69. [PubMed: 16467876]
6. Tenhumberg S, Schuster B, Zhu L, Kovaleva M, Scheller J, Kallen KJ, Rose-John S. gp130 dimerization in the absence of ligand: preformed cytokine receptor complexes. *Biochem Biophys Res Commun* 2006;346:649–657. [PubMed: 16774741]
7. Krause CD, Mei E, Mirochnitchenko O, Lavnikova N, Xie J, Jia Y, Hochstrasser RM, Pestka S. Interactions among the components of the interleukin-10 receptor complex. *Biochem Biophys Res Commun* 2006;340:377–385. [PubMed: 16364239]
8. de Bakker BI, Bodnar A, van Dijk EM, Vamosi G, Damjanovich S, Waldmann TA, van Hulst NF, Jenei A, Garcia-Parajo MF. Nanometer-scale organization of the alpha subunits of the receptors for IL2 and IL15 in human T lymphoma cells. *J Cell Sci* 2008;121:627–633. [PubMed: 18287585]
9. Jenei A, Kormos J, Szentesi G, Veres AJ, Varga S, Bodnar A, Damjanovich S, Matyus L. Non-random distribution of interleukin receptors on the cell surface. *Chemphyschem* 2009;10:1577–1585. [PubMed: 19514033]
10. Weidemann T, Hofinger S, Muller K, Auer M. Beyond dimerization: a membrane-dependent activation model for interleukin-4 receptor-mediated signalling. *J Mol Biol* 2007;366:1365–1373. [PubMed: 17223132]
11. Brown RJ, Adams JJ, Pelekanos RA, Wan Y, McKinsty WJ, Palethorpe K, Seeber RM, Monks TA, Eidne KA, Parker MW, Waters MJ. Model for growth hormone receptor activation based on subunit rotation within a receptor dimer. *Nat Struct Mol Biol* 2005;12:814–821. [PubMed: 16116438]
12. Kubatzky KF, Liu W, Goldgraben K, Simmerling C, Smith SO, Constantinescu SN. Structural requirements of the extracellular to transmembrane domain junction for erythropoietin receptor function. *J Biol Chem* 2005;280:14844–14854. [PubMed: 15657048]
13. Strunk JJ, Gregor I, Becker Y, Li Z, Gavutis M, Jaks E, Lamken P, Walz T, Enderlein J, Piehler J. Ligand binding induces a conformational change in ifnar1 that is propagated to its membrane-proximal domain. *J Mol Biol* 2008;377:725–739. [PubMed: 18294654]
14. Arkin MR, Whitty A. The road less traveled: modulating signal transduction enzymes by inhibiting their protein-protein interactions. *Curr Opin Chem Biol* 2009;13:284–290. [PubMed: 19553156]
15. Piehler J, Roisman LC, Schreiber G. New structural and functional aspects of the type I interferon-receptor interaction revealed by comprehensive mutational analysis of the binding interface. *J Biol Chem* 2000;275:40425–40433. [PubMed: 10984492]
16. Gavutis M, Jaks E, Lamken P, Piehler J. Determination of the two-dimensional interaction rate constants of a cytokine receptor complex. *Biophys J* 2006;90:3345–3355. [PubMed: 16473899]
17. Jaks E, Gavutis M, Uze G, Martal J, Piehler J. Differential receptor subunit affinities of type I interferons govern differential signal activation. *J Mol Biol* 2007;366:525–539. [PubMed: 17174979]
- 18*. Kalie E, Jaitin DA, Podoplelova Y, Piehler J, Schreiber G. The stability of the ternary interferon-receptor complex rather than the affinity to the individual subunits dictates differential biological activities. *J Biol Chem* 2008;283:32925–32936. In the interferon system, one of receptors is the high affinity cytokine binder, while the other receptor acts as the signaling molecule, binding interferon with low affinity. In this study it was investigated what will happen if the low affinity receptor would be engineered to be of high affinity, while the binding of the high affinity receptor was reduced to low affinity. The final conclusion was that the stability of the ternary complex drives the differential activities, independent on which receptor is the tighter binder. [PubMed: 18801736]
19. Rani MR, Pandalai S, Shrock J, Almasan A, Ransohoff RM. Requirement of catalytically active Tyk2 and accessory signals for the induction of TRAIL mRNA by IFN-beta. *J Interferon Cytokine Res* 2007;27:767–779. [PubMed: 17892398]
20. Vitale G, de HWW, van KPM, Waaijers M, Schoordijk W, Croze E, Colao A, Lamberts SW, Hofland LJ. IFN-beta is a highly potent inhibitor of gastroenteropancreatic neuroendocrine tumor cell growth in vitro. *Cancer Res* 2006;66:554–562. [PubMed: 16397272]

21. Vannucchi S, Chiantore MV, Fiorucci G, Percario ZA, Leone S, Affabris E, Romeo G. TRAIL is a key target in S-phase slowing-dependent apoptosis induced by interferon-beta in cervical carcinoma cells. *Oncogene* 2005;24:2536–2546. [PubMed: 15735750]
22. Marijanovic Z, Ragimbeau J, van der Heyden J, Uze G, Pellegrini S. Comparable potency of IFNalpha2 and IFNbeta on immediate JAK/STAT activation but differential down-regulation of IFNAR2. *Biochem J* 2007;407:141–151. [PubMed: 17627610]
23. Rani MR, Ransohoff RM. Alternative and accessory pathways in the regulation of IFN-beta-mediated gene expression. *J Interferon Cytokine Res* 2005;25:788–798. [PubMed: 16375607]
24. Pitha PM, Kunzi MS. Type I interferon: the ever unfolding story. *Curr Top Microbiol Immunol* 2007;316:41–70. [PubMed: 17969443]
25. Li Z, Strunk JJ, Lamken P, Piehler J, Walz T. The EM structure of a type I interferon-receptor complex reveals a novel mechanism for cytokine signaling. *J Mol Biol* 2008;377:715–724. [PubMed: 18252254]
- 26*. Pan M, Kalie E, Scaglione BJ, Raveche ES, Schreiber G, Langer JA. Mutation of the IFNAR-1 receptor binding site of human IFN-alpha2 generates type I IFN competitive antagonists. *Biochemistry* 2008;47:12018–12027. A ligand that binds multiple receptors can be transformed from agonist to antagonist by nullifying binding to one receptor, and increasing the affinity to the other receptor. This is what is shown here, resulting in a complete blockage of type I interferon activity. [PubMed: 18937499]
27. Akabayov SR, Biron Z, Lamken P, Piehler J, Anglister J. NMR mapping of the IFNAR1-EC binding site on IFNalpha2 reveals allosteric changes in the IFNAR2-EC binding site. *Biochemistry* 2010;49:687–695. [PubMed: 20047337]
28. Jaitin DA, Roisman LC, Jaks E, Gavutis M, Piehler J, Van der Heyden J, Uze G, Schreiber G. Inquiring into the differential action of interferons (IFNs): an IFN-alpha2 mutant with enhanced affinity to IFNAR1 is functionally similar to IFN-beta. *Mol Cell Biol* 2006;26:1888–1897. [PubMed: 16479007]
29. Kalie E, Jaitin DA, Abramovich R, Schreiber G. An Interferon {alpha}2 Mutant Optimized by Phage Display for IFNAR1 Binding Confers Specifically Enhanced Antitumor Activities. *J Biol Chem* 2007;282:11602–11611. [PubMed: 17310065]
30. Moraga I, Harari D, Schreiber G, Uze G, Pellegrini S. Receptor density is key to the alpha2/beta interferon differential activities. *Mol Cell Biol* 2009;29:4778–4787. [PubMed: 19564411]
31. Roisman LC, Jaitin DA, Baker DP, Schreiber G. Mutational Analysis of the IFNAR1 Binding Site on IFNalpha2 Reveals the Architecture of a Weak Ligand-Receptor Binding-site. *J Mol Biol* 2005;353:271–281. [PubMed: 16171819]
32. Thanos CD, DeLano WL, Wells JA. Hot-spot mimicry of a cytokine receptor by a small molecule. *Proc Natl Acad Sci U S A* 2006;103:15422–15427. [PubMed: 17032757]
33. Sato A, Sone S. A peptide mimetic of human interferon (IFN)-beta. *Biochem J* 2003;371:603–608. [PubMed: 12542398]
34. Ozes ON, Reiter Z, Klein S, Blatt LM, Taylor MW. A comparison of interferon-Con1 with natural recombinant interferons-alpha: antiviral, antiproliferative, and natural killer-inducing activities. *J Interferon Res* 1992;12:55–59. [PubMed: 1573283]
35. Yamamoto K, Taniai M, Torigoe K, Yamamoto S, Arai N, Suemoto Y, Yoshida K, Okura T, Mori T, Fujioka N, et al. Creation of Interferon-alpha8 (IFN-alpha8) Mutants with Amino Acid Substitutions Against IFN-alpha Receptor-2 (IFNAR-2)-Binding Sites Using Phage Display System and Evaluation of Their Biologic Properties. *J Interferon Cytokine Res* 2009;29:161–170. [PubMed: 19196068]
36. Benveniste EN, Qin H. Type I interferons as anti-inflammatory mediators. *Sci STKE* 2007;2007:pe70. [PubMed: 18073382]
37. Chen HM, Tanaka N, Mitani Y, Oda E, Nozawa H, Chen JZ, Yanai H, Negishi H, Choi MK, Iwasaki T, et al. Critical role for constitutive type I interferon signaling in the prevention of cellular transformation. *Cancer Sci.* 2008
38. Crow MK. Long interspersed nuclear elements (LINE-1): potential triggers of systemic autoimmune disease. *Autoimmunity* 2010;43:7–16. [PubMed: 19961365]

39. Crow YJ, Rehwinkel J. Aicardi-Goutieres syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity. *Hum Mol Genet* 2009;18:R130–6. [PubMed: 19808788]
40. Yao Y, Richman L, Higgs BW, Morehouse CA, de los Reyes M, Brohawn P, Zhang J, White B, Coyle AJ, Kiener PA, Jallal B. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. *Arthritis Rheum* 2009;60:1785–1796. [PubMed: 19479852]
41. Peleg-Shulman T, Roisman LC, Zupkovitz G, Schreiber G. Optimizing the Binding Affinity of a Carrier Protein: A case study on the interaction between soluble ifnar2 and interferon {beta}. *J Biol Chem* 2004;279:18046–18053. [PubMed: 14960565]
42. Randall RE, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol* 2008;89:1–47. [PubMed: 18089727]
43. Alcamí A, Symons JA, Smith GL. The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN. *J Virol* 2000;74:11230–11239. [PubMed: 11070021]
44. Waibler Z, Anzaghe M, Frenz T, Schwantes A, Pohlmann C, Ludwig H, Palomo-Otero M, Alcamí A, Sutter G, Kalinke U. Vaccinia virus-mediated inhibition of type I interferon responses is a multifactorial process involving the soluble type I interferon receptor B18 and intracellular components. *J Virol* 2009;83:1563–1571. [PubMed: 19073732]
45. Del Mar Fernandez de Marco M, Alejo A, Hudson P, Damon IK, Alcamí A. The highly virulent variola and monkeypox viruses express secreted inhibitors of type I interferon. *FASEB J*. 2009
46. Bello AM, Bende T, Wei L, Wang X, Majchrzak-Kita B, Fish EN, Kotra LP. De novo design of nonpeptidic compounds targeting the interactions between interferon-alpha and its cognate cell surface receptor. *J Med Chem* 2008;51:2734–2743. [PubMed: 18393399]
47. Goldman LA, Zafari M, Cutrone EC, Dang A, Brickelmeier M, Runkel L, Benjamin CD, Ling LE, Langer JA. Characterization of antihuman IFNAR-1 monoclonal antibodies: epitope localization and functional analysis. *J Interferon Cytokine Res* 1999;19:15–26. [PubMed: 10048764]
48. Slutzki M, Jaitin DA, Yehezkel TB, Schreiber G. Variations in the Unstructured C-terminal Tail of Interferons Contribute to Differential Receptor Binding and Biological Activity. *J Mol Biol* 2006;360:1019–1030. [PubMed: 16815442]
49. Walter MR, Windsor WT, Nagabhushan TL, Lundell DJ, Lunn CA, Zauodny PJ, Narula SK. Crystal structure of a complex between interferon-gamma and its soluble high-affinity receptor [see comments]. *Nature* 1995;376:230–235. [PubMed: 7617032]
50. Randal M, Kossiakoff AA. The structure and activity of a monomeric interferon-gamma:alpha-chain receptor signaling complex. *Structure* 2001;9:155–163. [PubMed: 11250200]
51. Sadir R, Forest E, Lortat-Jacob H. The heparan sulfate binding sequence of interferon-gamma increased the on rate of the interferon-gamma-interferon-gamma receptor complex formation. *J Biol Chem* 1998;273:10919–10925. [PubMed: 9556569]
52. Lortat-Jacob H, Baltzer F, Grimaud JA. Heparin decreases the blood clearance of interferon-gamma and increases its activity by limiting the processing of its carboxyl-terminal sequence. *J Biol Chem* 1996;271:16139–16143. [PubMed: 8663206]
- 53*. Nuara AA, Walter LJ, Logsdon NJ, Yoon SI, Jones BC, Schriewer JM, Buller RM, Walter MR. Structure and mechanism of IFN-gamma antagonism by an orthopoxvirus IFN-gamma-binding protein. *Proc Natl Acad Sci U S A* 2008;105:1861–1866. The paper reveals the surprising tetrameric structure of the poxvirus IFN- γ BP. Mutations in the tetramerization domain reveal its importance for IFN- γ neutralization. In addition, the IFN- γ BP binds to the positively charged IFN- γ C-terminal tail, which does not occur in the cellular IFN- γ /IFN- γ R1 complex. [PubMed: 18252829]
54. Reinisch W, Hommes DW, Van Assche G, Colombel JF, Gendre JP, Oldenburg B, Teml A, Geboes K, Ding H, Zhang L, et al. A dose escalating, placebo controlled, double blind, single dose and multidose, safety and tolerability study of fontolizumab, a humanised anti-interferon gamma antibody, in patients with moderate to severe Crohn's disease. *Gut* 2006;55:1138–1144. [PubMed: 16492717]

55. Thakur AB, Landolfi NF. A potent neutralizing monoclonal antibody can discriminate amongst IFN γ from various primates with greater specificity than can the human IFN γ receptor complex. *Mol Immunol* 1999;36:1107–1115. [PubMed: 10698312]
56. Landolfi NF, Thakur AB, Fu H, Vasquez M, Queen C, Tsurushita N. The integrity of the ball-and-socket joint between V and C domains is essential for complete activity of a humanized antibody. *J Immunol* 2001;166:1748–1754. [PubMed: 11160220]
57. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. *Immunol Rev* 2008;223:20–38. [PubMed: 18613828]
58. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009;27:519–550. [PubMed: 19302047]
59. Boraschi D, Tagliabue A. The interleukin-1 receptor family. *Vitam Horm* 2006;74:229–254. [PubMed: 17027517]
60. Luheshi NM, Rothwell NJ, Brough D. Dual functionality of interleukin-1 family cytokines: implications for anti-interleukin-1 therapy. *Br J Pharmacol* 2009;157:1318–1329. [PubMed: 19681864]
61. Dahlen E, Barchan K, Herrlander D, Hojman P, Karlsson M, Ljung L, Andersson M, Backman E, Hager AC, Walse B, et al. Development of interleukin-1 receptor antagonist mutants with enhanced antagonistic activity in vitro and improved therapeutic efficacy in collagen-induced arthritis. *J Immunotoxicol* 2008;5:189–199. [PubMed: 18569390]
62. Waldmann TA, O'Shea J. The use of antibodies against the IL-2 receptor in transplantation. *Curr Opin Immunol* 1998;10:507–512. [PubMed: 9794841]
63. Arkin MR, Randal M, DeLano WL, Hyde J, Luong TN, Oslob JD, Raphael DR, Taylor L, Wang J, McDowell RS, et al. Binding of small molecules to an adaptive protein-protein interface. *Proc Natl Acad Sci U S A* 2003;100:1603–1608. [PubMed: 12582206]
64. Ishino T, Pillalamarri U, Panarello D, Bhattacharya M, Urbina C, Horvat S, Sarkhel S, Jameson B, Chaiken I. Asymmetric usage of antagonist charged residues drives interleukin-5 receptor recruitment but is insufficient for receptor activation. *Biochemistry* 2006;45:1106–1115. [PubMed: 16430207]
65. Shen F, Gaffen SL. Structure-function relationships in the IL-17 receptor: implications for signal transduction and therapy. *Cytokine* 2008;41:92–104. [PubMed: 18178098]
- 66*. Gerhard S, Abbott WM, Hargreaves D, Pauptit RA, Davies RA, Needham MR, Langham C, Barker W, Aziz A, Snow MJ, et al. Structure of IL-17A in complex with a potent, fully human neutralizing antibody. *J Mol Biol* 2009;394:905–921. Here the authors describe the process of development of a neutralizing Ab, from primary selection through in vitro optimization to achieve higher binding affinity and finally the structural and functional characterization of the Ab bound to IL-17 and its mode of neutralization. [PubMed: 19835883]
67. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol* 2004;22:929–979. [PubMed: 15032600]
68. Bleicher L, de Moura PR, Watanabe L, Colau D, Dumoutier L, Renauld JC, Polikarpov I. Crystal structure of the IL-22/IL-22R1 complex and its implications for the IL-22 signaling mechanism. *FEBS Lett* 2008;582:2985–2992. [PubMed: 18675809]
- 69*. Jones BC, Logsdon NJ, Walter MR. Structure of IL-22 bound to its high-affinity IL-22R1 chain. *Structure* 2008;16:1333–1344. This paper elucidated the binding parameters of IL-22/IL-22R1 and IL-22/IL-22BP. This data, along with the structure of the IL-22/IL-22R1 complex, provide a structural mechanism for specificity of IL-10 cytokines. [PubMed: 18599299]
70. Josephson K, Logsdon NJ, Walter MR. Crystal structure of the IL-10/IL-10R1 complex reveals a shared receptor binding site. *Immunity* 2001;15:35–46. [PubMed: 11485736]
71. Josephson K, Jones BC, Walter LJ, DiGiacomo R, Indelicato SR, Walter MR. Noncompetitive antibody neutralization of IL-10 revealed by protein engineering and x-ray crystallography. *Structure* 2002;10:981–987. [PubMed: 12121653]
72. Logsdon NJ, Jones BC, Allman JC, Izotova L, Schwartz B, Pestka S, Walter MR. The IL-10R2 binding hot spot on IL-22 is located on the N-terminal helix and is dependent on N-linked glycosylation. *J Mol Biol* 2004;342:503–514. [PubMed: 15327950]

73. Boniface K, Guignouard E, Pedretti N, Garcia M, Delwail A, Bernard FX, Nau F, Guillet G, Dagregorio G, Yssel H, et al. A role for T cell-derived interleukin 22 in psoriatic skin inflammation. *Clin Exp Immunol* 2007;150:407–415. [PubMed: 17900301]
74. Li J, Tomkinson KN, Tan XY, Wu P, Yan G, Spaulding V, Deng B, Annis-Freeman B, Heveron K, Zollner R, et al. Temporal associations between interleukin 22 and the extracellular domains of IL-22R and IL-10R2. *Int Immunopharmacol* 2004;4:693–708. [PubMed: 15120653]
- 75*. de Moura PR, Watanabe L, Bleicher L, Colau D, Dumoutier L, Lemaire MM, Renauld JC, Polikarpov I. Crystal structure of a soluble decoy receptor IL-22BP bound to interleukin-22. *FEBS Lett* 2009;583:1072–1077. This manuscript provides the first look at the structure of the cellular high affinity IL-22BP bound to IL-22. [PubMed: 19285080]
76. Sheehan KC, Lai KS, Dunn GP, Bruce AT, Diamond MS, Heutel JD, Dongo-Arthur C, Carrero JA, White JM, Hertzog PJ, Schreiber RD. Blocking monoclonal antibodies specific for mouse IFN- α /beta receptor subunit 1 (IFNAR-1) from mice immunized by in vivo hydrodynamic transfection. *J Interferon Cytokine Res* 2006;26:804–819. [PubMed: 17115899]
77. Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, Pestka S. Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity. *J Immunol* 2001;166:7096–7103. [PubMed: 11390454]

Figure 1.
The different natural and engineered strategies employed to obtain agonist and antagonist cytokine activities.

Table 1

Agonists and antagonists of cytokines.

Cytokine	Receptors	Antagonist	agonist	Type of molecule	Mode of action	Ref
IFN α/β	IFNAR1/2	IFN α 2 mutant		protein	Enhanced binding to IFNAR2, no binding to IFNAR1	[26*]
IFN α/β	IFNAR1/2	MEDI-545		Antibody	High affinity binding to IFN α/β	[40]
IFN β	IFNAR1/2	IFNAR2 mutant		Extracellular receptor	High affinity binding to IFN β	[41]
IFN α/β	IFNAR1/2	IFN α/β BP		Viral protein	High affinity binding to IFN α/β	[44]
IFN α/β	IFNAR1/2	IRRP		Small molecule mimetic	IFNAR2 recognition peptide-mimetic	[46]
IFN α/β	IFNAR1/2	MAR1 mAbs		Antibody	mAb that blocks binding towards IFNAR1	[76]
IFN α/β	IFNAR1/2		IFN α 2 mutant	protein	Binds much tighter to IFNAR1	[29]
IFN- γ	IFN- γ R1, IFN- γ R2	HuZAF		mAb	Seq/structure-based humanization	[55]
IFN- γ	IFN- γ R1, IFN- γ R2	IFN- γ BP		Viral antagonist	Poxvirus IFN γ R1 mimic with novel structure	[53*]
IL-1	IL-1R1, IL-1RAcP	IL-1R α Anakinra		protein	Natural antagonist binds tightly to IL-1R1	[60]
IL-1	IL-1R1, IL-1RAcP	IL-1R α mutant		protein	A 10-fold higher affinity variant of IL-1R α	[61]
IL-1	IL-1R1, IL-1RAcP	IL-1R2		Decoy receptor	Natural antagonist competes on binding IL-1	[60]
IL-2	γ_c , IL-2R α , IL-2R β	SP4206		Small molecule	Binding IL-2, EC $_{50}$ =70 nM	[32]
IL-2	γ_c , IL-2R α , IL-2R β	Daelizumb		mAb	mAb binding tightly to IL-2R α	[62]
IL-5	β_c , IL-5R α	AF17121		Cyclic peptide	Binding IL-5R α , EC $_{50}$ =50 nM	[4*]
IL-17	IL-17RA, IL-17RC	(CAT-2200) mAb to IL-17		mAb	Binding IL-17, EC $_{50}$ =1 nM	[66*]
IL-18	IL-18R α , IL-18R β	IL-18BP		protein	Natural antagonist binds tightly to IL-18	[57]
IL-22	IL-22R1, IL-10R2	Ab02		mAb	Targets IL-10R2 binding	[74]
IL-22	IL-22R1, IL-10R2	Ab04		mAb	Targets IL-22/IL-22R1 interface	[74]
IL-22	IL-22R1, IL-10R2	Ab 356A11 humanized		mAb	<i>In vitro</i> selected pM Ab against IL-22	PCT/US 2007/00 4430
IL-22	IL-22R1, IL-10R2	IL-22BP		Cellular binding protein	Natural antagonist Binds IL-22 with pM affinity	[69*, 77]