Structural Features of the Interleukin-10 Family of Cytokines

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Abstract: The interleukin-10 (IL-10) family of cytokines includes IL-10, a number of its viral gene homologs, and eight recently discovered cellular cytokines (IL-19, IL-20, IL-22, IL-24, IL-26, IFN-λ1, IFN-λ2, IFN-λ3). IL-10 is an intercalated dimer consisting of two six-helix bundle domains. Signal transduction occurs when each domain of IL-10 binds to two receptor chains, IL-10R1 and IL-10R2. Viral homologs use the same IL-10 receptor system, while cellular homologs use their own receptors: three long receptor chains (IL-20R1, IL-22R1 and IFN-λ1R1) and two short receptor chains (IL-20R2 and IL-10R2). Most of the cellular homologs belong to the IL-19 subfamily of cytokines including IL-19, IL-20, IL-22 and IL-24. It is likely that IFN-λ1, IFN-λ2, and IFN-λ3 also belong to the same subfamily. All these proteins are monomers in solution. Crystal structures of IL-19 and IL-22 show that the molecules consist of seven helices (A-G) forming a seven-helix bundle with compact hydrophobic core inside. Structures of complexes of IL-10 and CMVIL-10 with an extracellular domain of high affinity receptor IL-10R1 (sIL-10R1) showed that ligand/receptor interactions are of mostly polar nature, with two hydrophobic patches around receptor residues Tyr43 and Phe143 at the top and bottom of the interface. The location and structure of the binding site for the second receptor chain are still unknown. It has also been shown that in the case of IL-19 and IL-20, IL-20R2 rather than IL-20R1 is a high-affinity receptor chain. This review summarizes all published three-dimensional structures of the cytokines representing the IL-10 family of homologs, including the IL-19 subfamily and their interaction with appropriate receptors.

Key Words: Cytokines, interleukin-10, ligand/receptor interactions, helix bundle, signal transduction.

INTRODUCTION

Interleukin-10 (IL-10) (reviewed in ref. [1]) is a fascinating cytokine first identified by its ability to stop immune response by inhibiting production of a number of cytokines. Due to its suppressor and inhibitor ability, IL-10 was first called a cytokine synthesis inhibitory factor [2, 3]. IL-10 also plays a role in proliferation and differentiation of B cells, T cells and mast cells. Based on its immunomodulating functions, IL-10 has been considered an attractive candidate for therapeutic applications for treatment of acute and chronic inflammation, autoimmunity, cancer and infectious disease (reviewed in ref. [4]). Biologically functional human IL-10 (hIL-10) is a 36 kDa dimer [2, 5, 6] consisting of two 160 amino acid residue-long polypeptide chains [7]. Initiation of the signal transduction occurs when IL-10 binds to two receptor chains, IL-10R1 [8] and IL-10R2 [9]. Both chains consist of extracellular, transmembrane and intracellular/cytoplasmic domains (the cytoplasmic domain of receptor chain 1 is much longer than that of chain 2), and belong to the class II or interferon receptor family [10, 11], characterized by the presence of two particular disulfide bridges and the absence of the so-called "WSXWS" motif in the C-terminal part of the extracellular domain.

Subsequently, a number of viral and cellular gene homologs of IL-10 have been discovered (reviewed in references [12-16]). This family of the homologs, which is now called an IL-10 family, can be divided into two major groups: viral homologs and cellular homologs.

Viral homologs were found in the genome of Epstein-Barr virus [3, 7, 17] (EBV), equine herpesvirus type 2 [18] (EHV2), Orf parapoxvirus [19, 20] (OV), human and simian cytomegaloviruses [21, 22] (CMV), and Yaba-like disease virus [23] (YV). Although the amino acid identity of these proteins with hIL-10 varies between 23% and 85% (Fig. **1**), they are dimers having three-dimensional structures very similar to IL-10, as has been shown by the crystal structures of EBVIL-10 and CMVIL-10 [24, 25]. The discovery of viral homologs of human IL-10 suggested that the analog of IL-10 could be used by parasites to escape/monitor host immune response, this is likely the case, however, no experimental evidence for the role of viral IL-10s in this process has been published yet.

Cellular homologs (Table **1**), including IL-19, [26] IL-20, [27] IL-22 [28, 29], IL-24 [30], IL-26 [31], IFN-λ1, IFN- λ2, and IFN- λ3 (IL-29, IL-28A, IL-28B) [32, 33], have a much lower amino acid sequence similarity with hIL-10 and differ from both IL-10 and from each other in their biological functions. Most of them are monomers, having structures somewhat similar to the structure of the IL-10 domain [34, 35]; the only exception is IL-26, which probably is a dimer [13, 31].

It is certain that the classification of cytokines (reviewed in ref. [36]) needs and can be improved since consecutive numbering is not the best way to do it. One of the ways to classify proteins is based on their aggregation state and three-dimensional structure. Based on the crystal structure of IL-19 [34] and IL-22 [35], it has been suggested that a new

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                                                  Helix A
hIL-10      ....SPGQGT...QSENSC...THFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNL.L
EBVIL-10    ................QC...DNFP....QMLRDLRDAFSRVKTFFQTKDEVDNL.L
OVIL-10     ....EYEESE...EDKQQCGSSSNFPASLPHMLRELRAAFGKVKTFFQMKDQLNSM.L
EHV2IL-10   ....DNKYDS...ESGDDC...PTLPTSLPHMLHELRAAFSRVKTFFQMKDQLDNM.L
CMVIL-10    SEEAKPATTTTIKNTKPQC...R..PEDYATRLQDLRVTFHRVKPTLQR.EDDYSV.W
YVIL-10     ..............SLN.C...GIEH....NELNNIKNIFFKVRNVVQADDVDHNLRI

            Helix B  Helix C                  Helix
HIL-10      LKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPDI.KAHVNSLGENLKTLRLR
EBVIL-10    LKESLLEDFKGYLGCQALSEMIQFYLEEVMPQAENQDPEA.KDHVNSLGENLKTLRLR
OVIL-10     LTQSLLDDFKGYLGCQALSEMIQFYLEEVMPQAENHGPDI.KEHVNSLGEKLKTLRLR
EHV2IL-10   LDGSLLEDFKGYLGCQALSEMIQFYLEEVMPQAENHSTDQEKDKVNSLGEKLKTLRVR
CMVIL-10    LDGTV...VKGCWGCSVMDWLLRRYLEIVFPAGDHVYPGL.KTELHSMRSTLESIYKD
YVIL-10     LTPALLNNITVSETCFFIYDMFELYLNDVFVKYTN..TALKLNILKSLSSVANNFLAI

            D      Helix E    Helix F
hIL-10      LRRCHRFLPCENKSKAVEQVKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTMKIRN
EBVIL-10    LRRCHRFLPCENKSKAVEQIKNAFNKLQEKGIYKAMSEFDIFINYIEAYMTIKAR.
OVIL-10     LRRCHRFLPCENKSKAVEQVKRVFNMLQERGVYKAMSEFDIFINYIESYMTTKM..
EHV2IL-10   LRRCHRFLPCENKSKAVEQVKSAFSKLQEKGVYKAMSEFDIFINYIEAYMTTKMKN
CMVIL-10    MRQCPLLG.C.GDKSVISRLSQEAERKSDNGTRKGLSELDTLFSRLEEYLHSR.K.
YVIL-10     FNKVKKRRVKKNNVNVLEIKKLLLIDNNCK...KLFSEIDIFLTWVMA....KI..
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Fig. (1). Amino acid sequence alignment of hIL-10 and viral IL-10s. Amino acid residues identical to hIL-10 are shown in bold, residues of hIL-10 involved in binding with sIL-10R1 are underlined. Helices A-F are marked based on the crystal structure of hIL-10.

Table 1. Cellular Homologs of Human IL-10

Cytokine (suggest. name)	% identity with hIL-10	Aggregation state	Receptor	Biological role
IL-10	100	Dimer	IL-10R1/IL-10R2	Immunosuppressive, anti- inflammatory
IL-19 (IL-10F1)	21	Monomer	IL-20R1/IL-20R2	Immune response
IL-20 (IL-10F2)	29	Monomer	IL-20R1/IL-20R2 IL-22R1/IL-20R2	Skin differentiation
IL-22 (IL-10F3)	26	Monomer	IL-22R1/IL-10R2 IL-22BP	Acute phase response in hepatocytes
IL-24 (IL-10F4)	24	Monomer	IL-20R1/IL-20R2 IL-22R1/IL-20R2	Growth inhibition of different tumor types
IL-26 (IL-10F5)	27	Dimer	IL-20R1/IL-10R2	Immune response
IFN-λ1 (IL-10F6)	Low	Monomer	IFN- λ1R1/IL-10R2	Antiviral
IFN- λ2 (IL-10F7)	Low	Monomer	IFN- λ1R1/IL-10R2	Antiviral
IFN- λ3 (IL-10F8)	Low	Monomer	IFN- λ1R1/IL-10R2	Antiviral

IL-19 subfamily of IL-10 homologs could be introduced [34]. The homologs will possess certain common structural features: the aggregation state is a monomer, belong to the group of long chain cytokines [37, 38] and instead of the long helix A, they have two short helices, A and B, separated by a short β-strand.

The purpose of this review is two fold. First, to highlight what is known about the structure and functions of this diverse family of cytokines and related proteins. Secondly, as many IL-10 related proteins have been discovered, it is now appropriate to suggest a nomenclature system that would allow us to differentiate the related proteins while still making their structural relationships clear.

MOLECULAR STRUCTURE OF IL-10 AND ITS VIRAL HOMOLOGS

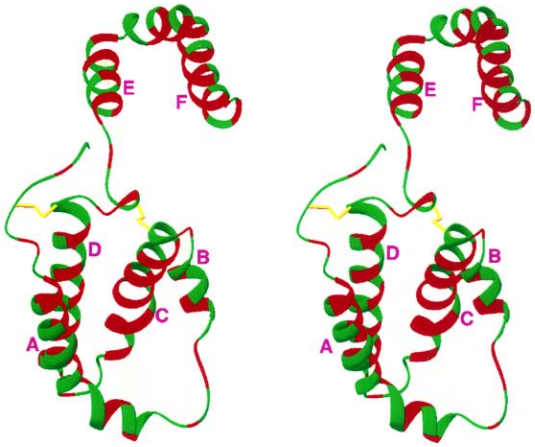
Structure of Human IL-10

IL-10 is an intercalated dimer of two subunits [39-41] consisting of six amphipathic helices A-F (Fig. **2a**). The polypeptide chains of each subunit contribute to both parts of the dimer. Helices A-D of one subunit form a distinctive six-helix domain with helices E' and F' through 180^{o} rotation around twofold axis (Fig. **2b**). In addition, helices A, C, D, F' and A', C', D' and F of each domain form a left-handed four-helix bundle, which was found in all helical cytokines [42]. The structure of the IL-10 subunit is stabilized by two intramolecular disulfide bridges (Fig. **2**), Cys12-Cys108 and Cys62-Cys114. Disulfides hold together helices A, C and D, forming a frame with a long depression in the middle. The internal surface of the frame is very hydrophobic; therefore, when amphipathic helices E' and F' cover the depression (Fig. **1b**), almost all (86%) hydrophobic residues of IL-10 are involved in formation of the intradomain hydrophobic core. Domains are kept together by two flexible polypeptide links, separated by 15 Å from each other, allowing some degree of freedom to change the elbow angle between the domains. A comparison of structures of hIL-10 crystallized in different crystal forms [39, 41] and EBVIL-10 [24], which is a very close homolog of hIL-10 (85% identity), indicated that the elbow angle may change easily, even due to a different crystal packing.

Monomeric IL-10

Exchange of structural elements between molecular aggregates is known as "domain swapping." A recent review [43] on domain swapping identified about 40 proteins, including IL-10, of known crystal structure which satisfy certain conditions for swapping. Theoretically, IL-10 has everything in order to swap helices E'-F' with E-F, which would lead to a formation of monomeric IL-10; however, in practice the disulfide bridge Cys62-Cys114 restricts possible conformations of loop DE, making it insufficiently long to allow the swapping without either reducing the disulfide or serious distortions of the structure [39, 41]. To overcome the problem, Josephson *et al*. [44] extended loop DE between Asn116 and Lys117 by insertion of an additional six amino acids (GlyGlyGlySerGlyGly) and showed that the expressed protein folded as a monomer. The crystal structure of monomeric IL-10 [45] confirmed that it is very similar to a single domain of the IL-10 dimer. Further analysis showed that it was able to form a 1:1 complex with IL-10 receptor 1 (IL-10R1); although affinity was 60-fold reduced but the protein still retained biological activity, which was 10-fold lower as compared with the dimeric wild-type IL-10. There was also a report [46] on the stability studies of the IL-10 dimer, which showed that dimer/monomer transition induced either by 1.6M guanidine hydrochloride or pH 2.5 did take place and that the monomer under such extreme conditions still retained 80% to 89% of its helical structure.

a).



b).

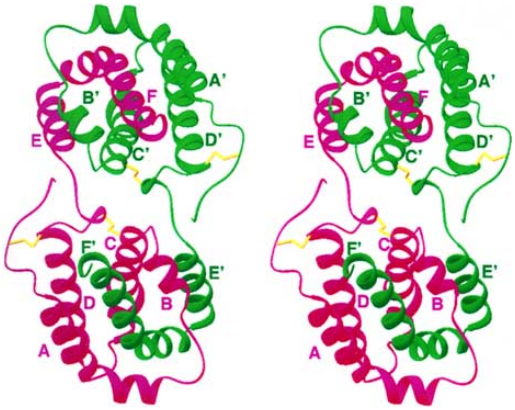


Fig. (2). a.) Stereo diagram of IL-10 monomer, hydrophobic residues marked in red (pdb code 2ILK); b.) Stereo diagram of IL-10 dimer, monomers are shown in violet and green. Disulfide bonds are in yellow. All figures are made with program RIBBONS [84].

Viral IL-10s

Since all viral homologs imitate at least a subset [47] of the biological function of IL-10, they initiate signal transduction through the IL-10 receptor system, by binding the high-affinity receptor chain IL-10R1 and the low-affinity chain IL-10R2, although the affinity of particular viral IL-10 may vary. For example, EBVIL-10 binding affinity toward IL-10R1 is about 1000-fold lower than that of human IL-10 [47], while CMVIL-10 has approximately the same binding affinity toward IL-10R1 as hIL-10 [25]. Yaba-like disease

virus homolog of IL-10 may be an exception among viral homologs of hIL-10, reference [15] quoted unpublished data specifying that this factor binds IL-20R1 and IL-20R2 receptor chains; however, since the homology of Yaba-like disease virus homolog is higher for IL-24 than for IL-10 (27% *vs* 23%), then this may actually be a viral homolog of IL-24, not of IL-10.

Two crystal structures of viral IL-10s have been determined to date, EBVIL-10 [24] at resolution 1.9 Å and CMVIL-10 [25] at resolution 2.7 Å; the latter structure has been solved in complex with IL-10 soluble receptor 1 (sIL-10R1). The amino acid sequence identity of each protein with hIL-10 is quite different, 85% for EBV and 27% for CMV IL-10 (Fig. **1**); because of that, the proteins have similar in general yet different in details three-dimensional structures (Fig. **3**), which are similar to IL-10. The subunit of EBVIL-10 consists of six helices, A-F, while the monomer of CMVIL-10 has only five helices. Because of three residues deletion in the area of short helix B, it is substituted by two extended β-turns. Thus, each domain of CMVIL-10 is formed by five helices; A, C, D of one subunit and E', F' coming from another subunit. The dimer of CMVIL-10 is additionally stabilized by a disulfide bond Cys59-Cys59' between the two subunits, making interdomain elbow angle 130° and restricting possible hinge movements of the domains. Unlike CMVIL-10, both human and EBVIL-10 elbow angles are in the range of about 90°, although they can change a few degrees even upon different packing of molecules in the crystals. The most significant difference of the amino acid sequences of EBVIL-10 and hIL-10 is found at the N-terminus. Because of the four residue deletion (17-20, hIL-10 numbering) the first disulfide bridge Cys12-Cys108 is shifted 6.1 Å away from its position in hIL-10, affecting the conformation of the surrounding areas, particularly that of loop DE [24]. Conformation of the N-terminus and loop DE in the structure of CMVIL-10 is also different from that of hIL-10 [25]. It was also noticed before that loop AB is likely to change its conformation upon interaction with the receptor [24]. This part of the structure was found relatively flexible in various crystal forms of hIL-10 [39-41] and adopts different conformation in EBVIL-10, in spite of the fact that amino acid sequence identity in this area is quite high [24]. Since the conformation of this loop in both receptor-bound hIL-10 and CMVIL-10 is the same and it is different from EBVIL-10, it is very likely that loop AB adopts its "active" conformation only upon interaction with the receptor.

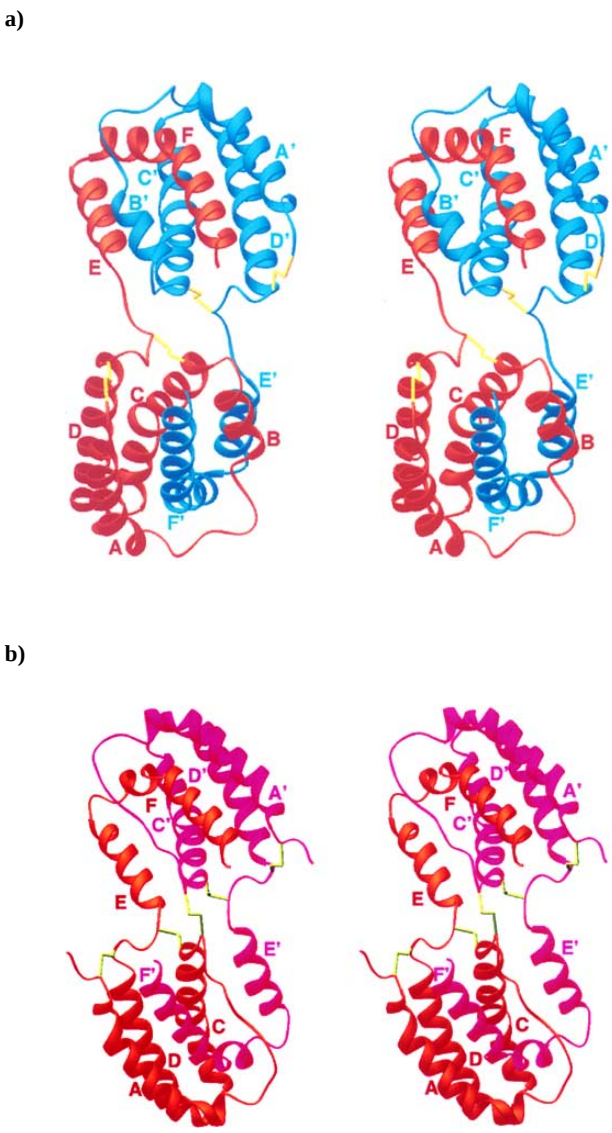


Fig. (3). a.) Stereo diagram of EBVIL-10 (pdb code 1VLK), monomers are in orange and cyan; b.) Stereo diagram of CMVIL-10 (pdb code 1LQS), monomers are in orange and violet.

LIGAND/SOLUBLE IL-10 RECEPTOR COMPLEXES

Stoichiometry of Ternary and Intermediate/Binary Complexes

It is commonly accepted that since IL-10R1 is the high-affinity receptor of IL-10, it should bind a ligand first [8], forming a binding site for the second receptor. The subsequent binding of the second receptor (IL-10R2) [9] completes the signaling ternary complex. Unfortunately, IL-10R2 is a low-affinity receptor [9, 48], and it is difficult, if not impossible, to obtain a stable ternary complex capable to crystallize. However, the binary intermediate complexes of both hIL-10 and CMVIL-10 with soluble receptor sIL-10R1 are quite stable and both were crystallized [49]. It was also shown that in solution IL-10 and sIL-10R1 form a complex consisting of two IL-10 dimers and four molecules of sIL-10R1 [50, 51]; therefore, the question of the exact stoichiometry of the signaling ternary complex is also open.

Crystals of a complex of hIL-10 [49] and CMVIL-10 with non-glycosylated mutant of the extracellular domain of IL-10R1 were obtained and both crystal structures were solved [25, 52] at resolution 2.9 Å and 2.7 Å, respectively. An asymmetric part of the crystal unit cell of the hIL-10 complex is composed of one domain of hIL-10 and one molecule of sIL-10R1. A rotation of 180° around the crystallographic twofold symmetry axis is required to form hIL-10 dimer bound to two sIL-10R1 molecules (Fig. **4**). In the case of the CMVIL-10 complex, an asymmetric unit

contains a CMVIL-10 dimer bound to two sIL-10R1 molecules. The receptor molecules interacting with the same IL-10 dimer do not interact with each other; the distance between their C-termini at the points of likely entrance into cell membrane is 110 Å and 105 Å for hIL-10 and CMVIL-10 complexes respectively.

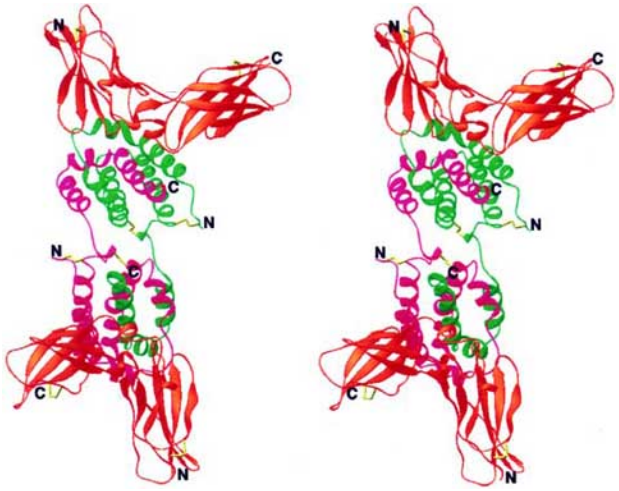


Fig. (4). Stereo diagram of hIL-10/sIL-10R1 complex (pdb code 1J7V) receptor molecules are in orange, IL-10 dimer has the same color code as in figure 1b.

Structure of Receptor Bound hIL-10

The structure of hIL-10 bound to its receptor is practically the same as found for free hIL-10, even the small change in the interdomain angle falls in the range of values found previously for human and viral IL-10s. The r.m.s. deviation for the Cα atoms of the parts of the molecule forming the helices is only 0.6 Å. Loops AB and DE, as well as N and C-termini, are more flexible and their conformations have greater differences. hIL-10 was previously crystallized in different crystal forms and at different temperatures; it is interesting that the structure of free hIL-10 crystallized in trigonal crystals and determined at temperature 100 K (pdb code 2ilk [40]) is the closest to the receptor-bound hIL-10 structure [52]. The reason for that is the unique packing of the molecules in the crystal unit cell, where symmetry related molecules of hIL-10 may serve as surrogates of the receptor molecules.

Structure of sIL-10R1

sIL-10R1 structure consists of N- and C-terminal domains (D1 and D2), each having fibronectin type III-like topology [25, 52, 53]. Each domain consists of two β sheets formed by seven antiparallel β strands A, B, E and G, F, C, C' (Fig. **5**), packed one against another in the form of a sandwich. The domains are comprised of residues 1-98, and 105-205, and are linked together by short linkage 99-104, consisting of one helical turn and three residue β strand L, which is hydrogen-bonded to both D1 and D2. Out of 14 loops connecting β strands, five loops located in the vicinity of the D1/D2 junction were found to be the most important [52] because they are involved in ligand receptor interactions. These are loops L2, L3 and L4 linking strands C-C', F-G, and G-L of the N-terminal domain D1 and L5, L6 linking strands B-C and F-G of the C-terminal domain D2 respectively (Fig. **5**). The structure is quite rigid, with no conformational changes found between sIL-10R1 molecules bound either to hIL-10 or CMVIL-10 [25].

Ligand/Receptor Interface Site Ia

It is interesting that in spite of the low amino acid sequence identity (27%) between hIL-10 and CMVIL-10, the interaction of either one with the receptor involves essentially the same areas on the surface of both the ligand and the receptor. In fact, a superposition of the CMVIL-10 domain bound to the sIL-10R1 with the structure of the hIL-10 domain bound to the sIL-10R1 gives an r.m.s. deviation only 1.2 Å for 331 Cα pairs. Most of the ligand/receptor contacts have a polar nature. A ligand/receptor interface is formed by

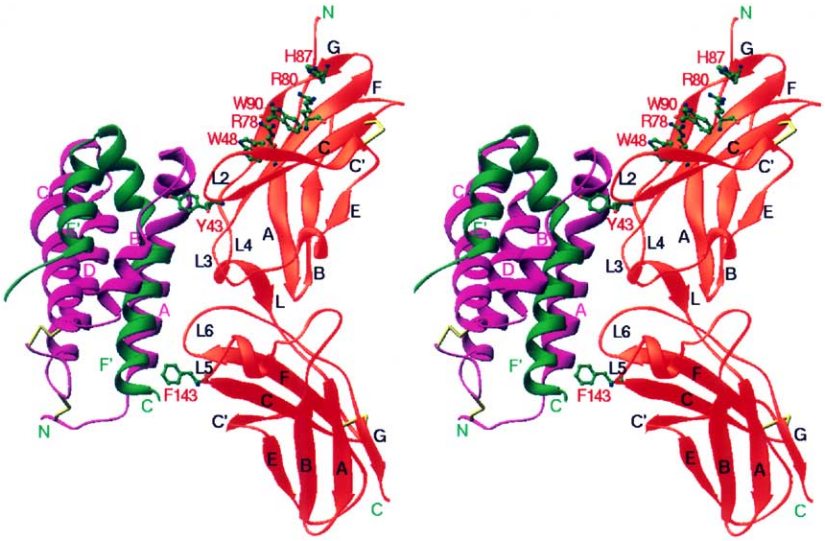


Fig. (5). Stereo diagram of one domain of hIL-10 bound to sIL-10R1, Tyr43 and Phe143 are shown to mark binding sites Ia and Ib, stack of Trp/Arg residues is also shown.

residues originating from helix A, interhelical loop AB and helix F' of the IL-10 and loops L2-L6 of the sIL-10R1, and it can be clustered into two interacting sites Ia and Ib [25, 52].

Site Ia includes the C-terminal part of helix A, loop AB, and the middle part of helix F on the IL-10 side and loops L2-L4 of the receptor, while site Ib includes the N-terminal and middle part of helix A, the C-terminal part of helix F of the IL-10, and loops L5-L6 of the receptor. Site Ia is the primary binding site, accounting for about 67% of the total buried surface of the interface [52]. It is centered around receptor residues Tyr43, Arg76 and Arg96, which make most of the interactions with IL-10. Tyr43 buries in the interface the most surface area of any residue involved in it (105 $Å^2$ and 110 $Å^2$ for hIL-10 and CMVIL-10 complexes, respectively) [25, 52]. In the complex with hIL-10, its hydroxyl group forms hydrogen bonds with the main chain carbonyl oxygen of Asn45, and side chains of Lys138 and Glu142, while its aromatic ring penetrates a hydrophobic cavity made by side chains of Leu46 and Ile145 of IL-10 and aliphatic parts of Arg76 and Arg96 of the receptor (Fig. **5**). In the CMV IL-10 complex, the side chain of Tyr43 rotates about 110° around the Cα-Cβ bond and in its new position makes bifurcated hydrogen bonds with the carboxyl oxygen of Asp42 of CMVIL-10 and a side-chain nitrogen of Arg76 of sIL-10R1. Its aromatic group is involved in a hydrophobic "face to edge" interaction with the side chain of Tyr44 (Asp in hIL-10) and Val46 (Leu in hIL-10) of the ligand and Leu41 and aliphatic parts of Arg76 and Arg96 of the receptor. The guanidino groups of Arg76 and Arg96 make extensive hydrogen bonds with the ligand. In the hIL-10 complex, the side chain of Arg76 adopts two alternative conformations: in the first one, it interacts with Asp44 and Gln42; in the second conformation, it interacts with main chain carbonyl oxygen of Gln38, and through bridging water Wat103 with Gln42. In the CMVIL-10 complex, only the second conformer of Arg76 is possible, because of the side chain of Tyr44 of CMVIL-10, which takes the space of the first conformer. Except for the interaction with Wat103, which is absent in the CMVIL-10 complex, Arg76 makes similar contacts with the ligand: main chain oxygen of Gln38 and hydroxyl of Tyr44 (Asp44). Arg96 has slightly different conformations in hIL-10 and CMVIL-10 complexes, affecting its interactions with the corresponding IL-10. In the hIL-10 complex, the NH1 atom makes hydrogen bonds to Gln38 and the carbonyl oxygen of Ser141, while its NH2 interacts with the carboxyl group of Asp144, and through bridging water Wat71 with Gln38 and Lys34. There is also a bridging water Wat88 in the site Ia mediating interactions of hIL-10 Lys34 with the carbonyl oxygen of Arg96, main chain nitrogen and hydroxyl group of Ser98 of the receptor. It is interesting that out of three water molecules involved in the ligand/receptor interface site Ia of the hIL-10 complex, Wat88 and Wat103 certainly belong to the receptor molecule, while Wat71 is likely to belong to free hIL-10, since it has a counterpart there—water molecule Wat260 (pdb entry 2ILK); the distance between the positions of Wat71 and Wat260 is only 1.7 Å, when free and receptor-bound IL-10 molecules are superimposed. In the CMVIL-10 complex, the NH1 atom of Arg96 makes a hydrogen bond to the carbonyl oxygen of Asn73 of the receptor, NH2 atom makes a hydrogen bond to the carbonyl of Ser141 and a weak hydrogen bond to the hydroxyl of Thr145, while the NE atom is hydrogen-bonded to the carboxyl of Asp144. There is also a possibility of an ionic interaction of the guanidino group with Glu142 (the shortest distance between the side chains of Glu142 and Arg96 is 4.3 Å). There are no water molecules in the vicinity of Arg96 in the CMVIL-10/sIL-10R1 interface.

Ligand/Receptor Interface Site Ib

Site Ib may be considered a secondary site; it is centered around IL-10 residues Arg27 and Glu151. In both complexes, guanidino group of Arg27 makes hydrogen bonds with the side chains of Asn148 (Ser148 in CMVIL-10), Glu151 of the ligand, and the carbonyl oxygen of Ser190 of the receptor. In addition, the carboxyl group of Glu151 interacts with the hydroxyl group of Ser190 and the side chain of Arg191 on the receptor side. However, in the case of CMVIL-10, interaction between Glu151 and Arg191 is purely ionic; corresponding distances are in the range of 4.2 to 4.4 Å. The side chain of Arg24 of hIL-10 also makes a hydrogen bond through its NE atom with the main chain carbonyl of Arg191. In CMVIL-10, where Arg24 is substituted by Gln24, the same hydrogen bond is formed by its NE2 atom. In the hIL-10/sIL-10R1 complex, hydrophobic contacts occur between the aromatic ring of the receptor Phe143, sandwiched between Pro20 and Ile158. In CMVIL-10, Pro20 is mutated to Ala20 and Ile158 is deleted. However, Pro16 of the CMVIL-10 took position in the vicinity of hIL-10 Ile158; in addition, His155, which substituted Thr155, makes a face-to-face interaction (corresponding distances are in the range of 3.3 to 3.6 Å) with His142 of the receptor (Fig. **6**). In other words, the hydrophobic interactions around Phe143 in the CMVIL-10/sIL-10R1 complex include not only not very strong contacts with Pro16 and Ala20, but also a His142 *vs* His155 pair, which also contributes to the same hydrophobic cluster (Fig. **6**).

Key Residues in Ligand/Receptor Interaction

Therefore, even though the majority of the interactions in the ligand/receptor interface have a polar nature, Tyr43 and Phe143 of the receptor represent two hydrophobic residues, located at the top and bottom of the ligand/receptor interface, which play the role of hydrophobic locks, keeping molecules together after they recognize each other by long-range ionic interactions. In the case of CMVIL-10, the hydrophobic interactions around Phe143 are enforced by additional face-to-face interaction of His155 of the ligand with His142 of the receptor.

Jones *et al*. [25] proposed a putative energetic hot spot based on amino acid sequence conservation between hIL-10 and viral homologs binding sIL-10R1 and the structural analysis of hIL-10/sIL-10R1 and CMVIL-10/sIL-10R1 complexes. These include eight residues of the ligand (Arg24 or Gln24, Arg27, Lys34, Gln38, Asp44 or Tyr44, Ser141, Asp144, and Glu151) and six residues of the receptor (Gly44, Arg76, Arg96, Glu101, Ser190, and Arg191).

It is interesting that EBVIL-10, having a very high identity (85%) toward hIL-10 [3, 7, 17], has about 1000-fold lower affinity toward IL-10R1 [1]. The crystal structure of the hIL-10/sIL-10R1 complex appears to explain why this is

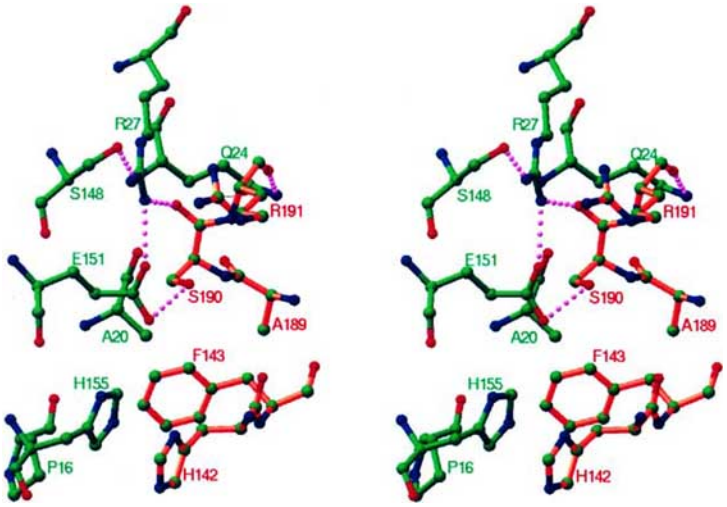


Fig. (6). Stereo diagram of interface site Ib of CMVIL-10/sIL-10R1 complex (pdb code 1LQS), CMVIL-10 residues are shown in green, receptor residues are in red, atom color code is: C-green, N-blue, O-red; hydrogen bonds are shown as pink dash lines.

so. The hydrophobic pocket made by hIL-10 residues Pro20 and Ile158 for receptor side chain of Phe143 does not exist in EBVIL-10: residues 17-20 are deleted, and Ile158 of hIL-10 is substituted by Ala. Because of the deletions at the N-terminus, the conformation of the main chain in this area is quite different and in order for Phe143 of the receptor to get into even weak hydrophobic contacts with IL-10, some local conformational changes on the ligand side have to occur. In addition, the conformation of the loop AB involved in formation of the site Ia is also different from what was found in the structure of EBVIL-10 [24]. These conformational changes would inevitably require additional energetic expense, lowering ligand/receptor affinity.

WSXWS Motif

Class I receptors have "WSXWS" motif in the C-terminal part of their extracellular domain, usually it is located in the β bulge preceding strand G. Indole rings of the tryptophans are oriented toward strand F and are intercalated with guanidino groups of two arginines coming from either strand C as in the structure of prolactin and erythropoietin receptors [54, 55] or strand F as in the structures of growth gormone and IL-4 receptors [56, 57] creating a stack of Trp/Arg/Trp/Arg residues forming an extended Π-cation system. It is interesting that Trp40, Arg78, Trp90 and Arg80 of the IL-10R1 form similar stack of Trp/Arg residues extended by Leu41 and His87 (Fig. **5**). Unlike class I receptors, it is located in the N-terminal domain of the sIL-10R1 in the vicinity of a "WSXWS"-like motif consisting of residues His87, Ser88, Asn89, Trp90 and Thr91 [52]. The role of the motif has been extensively studied by mutagenesis of the erythropoietin receptor [58, 59]. The obtained results have shown that the "WSXWS" motif was important for the passage of the receptor from endoplasmic reticulum to the Golgi apparatus.

Possible IL-10R2 Binding Sites

As mentioned above, in the solution hIL-10/sIL-10R1 complex is formed by two dimers of IL-10 and four molecules of the sIL-10R1 [50]; that is, 2:4 stoichiometry. It is obvious that *in vivo*, on the cell membrane the signaling complex could be different; nevertheless, it could also be 2:4. An asymmetric part of the crystal unit cell of the hIL-10/sIL-10R1 complex contains one monomer of hIL-10 and one molecule of sIL-10R1. A complex of hIL-10 dimer bound to two sIL-10R1 (1:2) is formed because of the crystallographic symmetry; if we continue this process one step further and apply translational symmetry along unit cell axis *b*, we can generate a 2:4 complex. This kind of complex is stabilized in the crystals [52] by interactions of the D1 domain of one molecule of the 1:2 complex with IL-10 and the D2 domain of the symmetry-mate molecule of the 1:2 complex. Josephson *et al*. [52] suggested that this could be the complex which was seen in the solution [50, 60] and it gave them a clue of how the signaling ternary complex, a complex of hIL-10 with both IL-10R1 and IL-10R2, may be organized. Since the key amino acids involved in the hIL-10/sIL-10R1 interactions in site Ia and Ib are mostly conserved between IL-10R1 and IL-10R2, they proposed an idea that at the first step both IL-10R1 and IL-20R2 may bind hIL-10 by using the same site I, so that there will be a mixture of high affinity hIL-10/IL-10R1 1:2 complexes and low-affinity hIL-10/IL-10R2 1:2 complexes. In the next step, these complexes interact with each other to produce a ternary (2:4) complex similar to one generated in the crystals through translational symmetry, and that one could be the signaling complex [60]. It is clear that when a receptor is bound to the membrane, then even weak interactions could become meaningful and from this point of view the events described above could be real. However, while in solution the hIL-10/sIL-10R1 complex was found to be 2:4 with a high degree of probability, the situation in the crystal is quite different. Crystal packing of IL-10/sIL-10R1 is such that ligand molecules make an infinite number of layers parallel to the plane *ab*, separated by parallel layers of the sIL-10R1 molecules. If we assume that the 1:2 complex lying on the twofold symmetry axis is a minimal unit, then millions of such units interact with each other, creating not just 2:4 but a continuous number of infinite layers of 1:2 molecules interacting exactly the same way as in the 2:4 molecule. Thus, if 1:2 molecules are favored in solution to form the 2:4

complexes found in the crystal, they inevitably must form higher aggregates in solution as well. Besides, a similar complex [25] of CMV IL-10 with the same non-glycosilated mutant of sIL-10R1 has an absolutely different crystal packing, and even though the 1:2 complex is very similar to hIL-10/sIL-10R1 both in terms of the structure of the receptor and its interaction with the ligand [25], there is no 2:4 CMV IL-10/sIL-10R1 complex found in solution. Therefore, it appears that the questions of the position of the IL-10R2 binding site and the structure of the ternary hIL-10/IL-10R1/IL-10R2 complex still remain to be answered.

IL-19 SUBFAMILY OF CYTOKINES

All members of the IL-19 subfamily of cytokines (IL-19, IL-20, IL-22, IL-24) are monomers in solution [34, 35, 61, 62], although at high concentration some aggregation may take place [35]. Despite their relatively low amino acid sequence identity with hIL-10, their structures, as will be seen below, are similar to the structure of one domain of hIL-10. Biological functions of these factors, particularly for their therapeutic applications, are still to be studied; however, some of the proteins have already attracted considerable interest. For instance, IL-24 (formerly known as MDA-7) has shown profound antiproliferative and cytotoxic effects in a wide variety of human tumor cell lines [62-69], and it is now becoming a subject of growing number of studies.

Crystal Structure of IL-19

A molecule of IL-19 is a monomer made up of seven amphipathic helices A-G of different lengths (Fig. **7a**), forming a unique seven-helix bundle with an extensive internal hydrophobic core. Three disulfide bridges located on the top of the bundle make the polypeptide chain framework quite rigid. Helices B, D, E and G make a four-helix bundle, which is a characteristic feature of all helical cytokines [42]. The position of helix A, covering the top of the molecule, is stabilized by the disulfide bridge Cys10-Cys103, linking it covalently to the C terminus of helix E. The second and third disulfide bridges, Cys57-Cys109 and Cys58-Cys111, hold together the N terminus of helix D, interhelical loop EF, and the N terminus of helix F. The C-terminal strand 154-159 is bent along the surface of the molecule and makes hydrogen bonds with the short interhelical strand AB. These two parallel strands form a short β-sheet, never seen previously in helical cytokines (Fig. **7a**).

Crystal Structure of IL-22

The crystal structure of IL-22 [35, 70] is topologically very similar to IL-19 (Fig. **7b**), although the sequence similarity between IL-19 and IL-22 is 36%, which is not as high as with IL-20 or IL-24. The structure also consists of seven α-helices A-G, which are packed as a seven-helix bundle having an extensive hydrophobic core inside. A superposition of the structures of IL-19 and IL-22 [35] results in r.m.s. deviation of 1.7 Å for 123 pairs of Cα-atoms. The main difference is in the position of the disulfide bridges and lengths of the loops. While the disulfide Cys10-Cys103 of IL-19 corresponds to Cys7-Cys99 of IL-22, they are also significantly shifted (2.5–5.5 Å for the respective

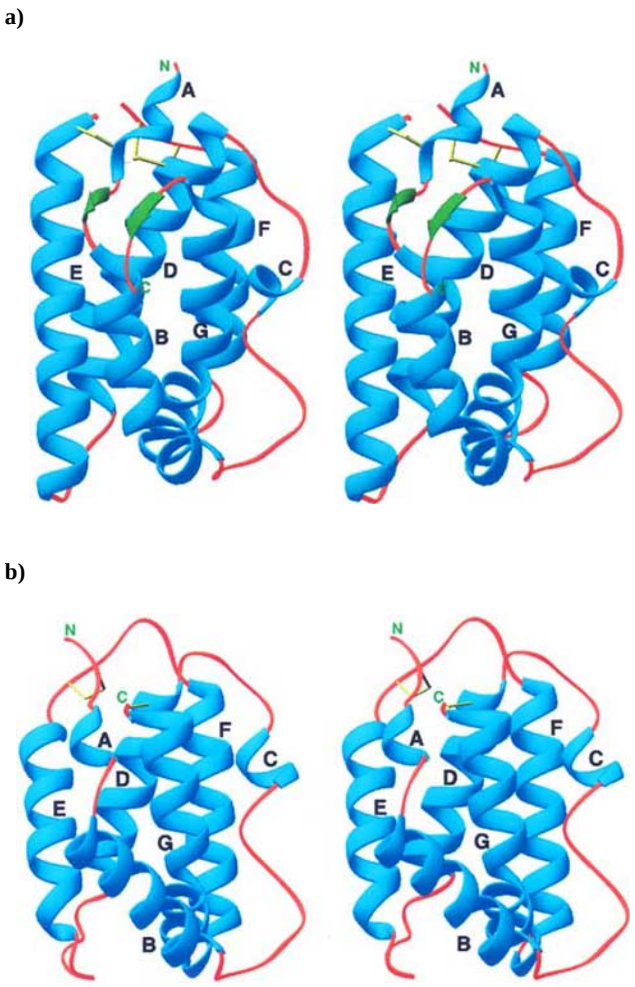


Fig. (7). a.) Stereo diagram of IL-19 (pdb code 1N1F); b.) Stereo diagram of IL-22 (pdb code 1MR4).

Cα coordinates). Cys57 of IL-19 is equivalent in sequence to Cys56 of IL-22; their Cα coordinates are only 3.3 Å apart. However, their disulfide partners are different: in IL-19, Cys57 makes a disulfide bond to Cys109 (loop EF); in IL-22, Cys56 makes a bond to Cys145, which is the C terminus of helix G. A similar variability of the disulfides has previously been reported for short-chain helical cytokines [38]. Therefore, in the IL-19 structure, the seven-helix bundle is stabilized by disulfides holding together N-the terminus of helix A with the C terminus of helix E, and the N terminus of helix D with both loop EF and the N terminus of helix F; while in IL-22, the first disulfide similar to IL-19 also holds together the N terminus of helix A and the C terminus of helix E, but the second disulfide is between the N terminus of helix D and the C terminus of helix G.

Comparison of IL-19 and IL-22 with IL-10

The superposition of IL-19 and IL-22 with one domain of IL-10 gives an r.m.s. deviation between the positions of Cα atoms 1.7 Å and 1.9 Å [35], respectively. The main differences are in the area of the first 21 residues of the IL-10 domain, helix C, interhelical loops, orientation of helix E

(helix D of IL-10) relative to the rest of the helical bundle, with the r.m.s. deviation at its N-terminus about 1 Å, increasing to 3.8 Å at the C terminus, and the C-terminus of helix G (helix F of the IL-10). Therefore, the general architecture of these molecules is very much alike, although the orientation of the new helix A and the short β-sheet in the IL-19 make the overall shape of the molecule more compact and smooth.

Interleukin-20

BestFit [71] comparison of IL-19 and IL-20 gives 44.1% sequence identity and 52.4% similarity without any gaps or deletions, starting in the sequence at the position corresponding to the N terminus of helix A of IL-19. The superposition of the sequences of IL-19, IL-20 and IL-10 shows a remarkable degree of similarity in the positions of hydrophobic residues and of the cysteines involved in the formation of the disulfide bridges. In fact, all three disulfide bonds present in IL-19 are also preserved in IL-20. Taken together, we must conclude that the three-dimensional structure of IL-20 must be similar to IL-19, and it is no surprise that these two cytokines share their receptors. The only obvious difference between IL-19 and IL-20, besides their N termini, is expected at the C terminus, in the region of the C-terminal β-strand of IL-19. There is no such strand in IL-20 since Glu157, the last residue of IL-20, corresponds to His153 of IL-19, which is the last residue in the helix G.

Interleukin-24

Sequence identity between IL-19 and IL-24 (MDA-7) is slightly lower, 31%, with 40% similarity. However, allocation of similar and hydrophobic amino acid residues confirms that the three-dimensional structure of IL-24 is likely to be similar to that of IL-19. Interestingly, IL-24 has only two cysteines (Cys16 and Cys63), corresponding to Cys10 and Cys57 of IL-19. Since these two cysteines are involved in making separate disulfide bonds, and IL-24 is a monomer, this must indicate that the N terminus of IL-24 should have a unique conformation, which is likely to bring Cys16 into the proximity of Cys63 to form a disulfide bridge between them. Because of that, the position of short helix A relative to the rest of the helical bundle may be different and may also affect binding to the receptor. Another structural feature, which is highly conserved between these cytokines, is a salt bridge formed between Lys27 and Asp143 and located on the surface of the molecule. This bridge is strictly conserved in IL-19, IL-10, IL-22 and IL-24, with a mutation to Arg in IL-20.

Putative Ligand/Receptor Complexes

Cellular homologs, including the IL-19 subfamily, employ different from IL-10 receptor systems, although they very often overlap and IL-10R2 is sometimes utilized as the second receptor chain, but IL-10R1 is never used. IL-20R1, IL-20R2 [27, 72, 73], IL-22R1 [29, 74] and IFN-λ1R1 [32, 33] have been discovered, along with their ligands. Except for IL-20R2, all other newly discovered receptors have long cytoplasmic domains; in fact, because of that, they are called the first receptor chains. Therefore, eight cytokines signal by using one of three first receptor chains in combination with one of two second receptor chains (IL-20R2 or IL-10R2). IL-22 also has a natural soluble receptor or IL-22 binding protein (IL-22BP), which is also a type II receptor, but it lacks both the transmembrane and intracellular domains [75-77] and binds IL-22 on its own.

It was shown that IL-19, IL-20 and IL-24 signal through the same two chains, IL-20R1 and IL-20R2 [61, 78]; in addition, IL-20 and IL-24 signal through the pair IL-22R1/IL-20R2 [65, 72, 73] and IL-22 uses IL-22R1 and IL-10R2 [28, 74]. It was shown recently that despite what had been commonly accepted, IL-20R2 is a high-affinity receptor chain in the case of IL-19 and IL-20. It binds the ligand first [61], forming a binding site for the long receptor chain IL-20R1, which binds the second. It is very likely that IL-24 also binds to IL-20R2 first. Since the three-dimensional structure of these cytokines is similar to the structure of one domain of IL-10 and all receptors belong to the same family, it is reasonable to assume that receptor binding sites should be also somewhat similar. The simple superposition of IL-19 onto one domain of IL-10 bound to sIL-10R1 [52] allowed to mark the IL-19 surface with amino acid residues which may potentially interact with the receptor [34]; these included helix B, loop BC and helix G. No crystal structures of any of the IL-19 subfamily ligand/receptor complexes are available at present; however, a model of the complex of IL-22 with sIL-22R1, generated on the basis of the crystal structure of the complex of hIL-10/sIL-10R1, was published [79]. Not only was the sIL-22R1 structure generated in this model, but IL-22 was modeled, too, since no crystal structure of IL-22 was at that time available. Nevertheless, amino acid residues which might be involved in specific contacts with the receptor were identified and they were essentially the same as the ones found with the help of the crystal structure of IL-22 [35]. It is interesting to note that intermolecular interface in a dimer of IL-22 molecules in the asymmetric part of the crystal unit cell included a receptor binding site with one of the arginines playing the role of Arg-96 of the receptor [35], making extensive hydrogen bonds similar to those found in the hIL-10/sIL-10R1 complex.

Interleukin-26

IL-26 appears to be the only cellular homolog of IL-10 which could be a dimer [31]. Amino acid sequence analysis [71] of IL-26 gives 31% identity and 45% similarity with hIL-10, which is slightly higher than with IL-19 (27% and 35%), with IL-22 (27% and 38%), or with IL-24 (30% and 37%). It is obvious that its structure should be similar to IL-10; however, it still remains to be seen to what degree. Another feature of IL-26 is that it has a very high isoelectric point of 10.8, which is quite unusual for the family of IL-10 homologs, which more often have isoelectric points in the range of 7 to 8. It is not clear why IL-26 needs to be positively charged. The protein was also for a while a "mystery cytokine" because no receptor system was identified for it. Recently it has been shown[1] that IL-26 shares receptors with other members of the IL-10 family. IL-26 employs IL-20R1 as the first receptor chain and IL-10R2 as the second one;

[1] Faruk Sheik, Vitaliy V. Baurin, Anita Lewis-Antes, Nital K. Shah, Sergey V. Smirnov, Shubha Anantha, Harold Dickensheets, Laure Dumoutier, Jean-Christophe Renauld, Alexander Zdanov, Raymond P. Donnelly and Sergei V. Kotenko, J. of Immunology, in press.

none of the other cellular homologs of IL-10 use this particular receptor pair. However, the question of whether or not IL-26 belongs to the IL-19 subfamily is still open.

NEW ARRIVALS, IFN- λ1, IFN- λ2, IFN- λ3 (IL-29, IL-28A AND IL-28B)

IFN-λ1, IFN-λ2, IFN-λ3 (IL-29, IL-28A and IL-28B) are recently discovered cytokines, having low sequence identity with IL-10 (10%-13%); nevertheless, all three were recognized as likely to belong to the family of IL-10 homologs [32, 33]. Sequence identity between IFN-λ1 and IFN-λ2 is 81%, and between IFN-λ2 and IFN-λ3, 96%. The proteins are monomers in solution (unpublished data) and signal through a newly discovered receptor chain, IFN-λ1R1, in pair with IL-10R2 [32, 33]. In fact, sequence identity between IFN-λs and IFN-α2 is slightly higher [33], particularly in terms of the positions of cysteine residues, which implies that IFN-λ1, IFN-λ2 and IFN-λ3 may have the same disulfide bridges as IFN-α2 (IFN-α2 and IFN-λ1 have five cysteines, IFN-λ2 and IFN-λ3 have seven). The three-dimensional structure of IFN-λ1, IFN-λ2 or IFN-λ3 has not yet been determined; however, it is possible that it will be similar to IL-19 subfamily, since the first helix of IFN-α2 [80] is shorter than that of IL-10 and somewhat similar to helix B of IL-19.

A NEW NOMENCLATURE FOR CELLULAR HOMOLOGS OF IL-10

As it has been already discussed, the classification of cytokines certainly needs to be improved, particularly in the cases of families of homologs, like IL-10. Since viral homologs of IL-10 always imitate the function and the structure of IL-10, there is no necessity to change anything with their names. In other words, EBVIL-10 or "the name of the virus" plus "IL-10" is clear enough, however, cellular homologs of IL-10 could be renamed in a way clearly showing what family of proteins or homologs they belong in. One of the way to do it has been suggested a couple years ago for the IL-1 family [81], and it can be easily adapted for IL-10 family. The name of each cellular homolog would be IL-10Fn, where IL-10 portion stays for IL-10, then letter "F" for the word "family" and number "n" is an order number of the publication/discovery of particular protein. For example, IL-19 would be IL-10F1, IL-20 would be IL-10F2 and so on. Suggested new names of all cellular homologs of IL-10 are shown in Table **1** in brackets as suggested names. The names of the corresponding receptors could be formed similarly as it was before, by adding letter "R" and chain number at the end of the name of the ligand, for example, IL-20R1 would become IL-10F2R1 and so on. IL-19 subfamily of cytokines would become IL-10F1 subfamily, which potentially may include all cellular homologs of IL-10 provided their three-dimensional structures satisfy the described above criteria.

CONCLUSIONS: CORRELATION OF STRUCTURE WITH FUNCTION

IL-10 is an intercalated dimer which initiates signal transduction through binding to two receptor chains in a sequential order. Initially, IL-10 binds IL-10R1, having a long cytoplasmic domain; as a result, the binding site for the second receptor chain, IL-10R2, is formed and its binding completes creation of the signaling complex.

The IL-10 family of cytokines can be divided into two major groups: viral and cellular homologs of IL-10. Viral homologs, designed to imitate the IL-10 function, bind to the same receptor system as IL-10 and necessarily must have a three-dimensional structure very similar to IL-10. Crystal structures of the complexes of hIL-10/sIL-10R1 and CMVIL-10/sIL-10R1 have proved that receptor moiety does not change its structure upon different ligand binding; however, ligands may encounter local conformational changes, particularly in the areas of the N-terminus and the inter-helical loop AB. Most of the contacts in the ligand/receptor interface are of a polar nature, although two areas around receptor amino acid residues Tyr43 and Phe143 serve as hydrophobic locks after ligand/receptor recognition has occurred. Since some of the viral homologs of IL-10 possess only a subset of the activities of IL-10, it is not quite clear how that can be accomplished with the help of the same receptor system. It might be suggested that affinity modulation may play important role in a case of somewhat lower expression of IL-10R1, since IL-10R2 is constitutively expressed in most tissues [82, 83]. However, the binding site and the mode of binding for the second receptor chain, IL-10R2, still remain to be found.

Most of the cellular members of the IL-10 family belong to the IL-19 subfamily, including IL-19, IL-20, IL-22 and IL-24. Even though the crystal structures of IFN-λ1, IFN-λ2 and IFN- λ3 have not been determined, it is likely that they also belong to the IL-19 subfamily. However, classification for IL-26 is not clear because of its aggregation state, even though the receptor system for this cytokine overlaps with the members of IL-19 subfamily. All eight members (Table **1**) of cellular homologs of IL-10 signal with the help of three long chain receptor chains (first receptors) and two short receptor chains (second receptors), in addition, IL-20 and IL-24 each use two different combinations of these receptors. Since the biological functions of these cytokines are different, an idea of tissue-specific expression of appropriate receptors is quite plausible. Size exclusion chromatography studies showed that IL-20R2 but not IL-20R1 is a high-affinity receptor of IL-19 and IL-20, and formation of a signaling ternary complex is likely to be a two-step procedure: initially, a ligand binds to IL-20R2, forming the binding site for IL-20R1, the binding of which completes the final complex. The high-affinity receptor binding sites, including helix B, loop BC and helix G, on the surface of IL-19 and IL-22 were marked, based on the known crystal structure of IL-10/sIL-10R1.

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