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Molecular modeling of the interleukin-19 receptor complex

Novel aspects of receptor recognition in the interleukin-10 cytokine family

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Abstract The interleukin-10 (IL-10) cytokine family consists of several viral and human homologs that exhibit distinct receptor binding specificities. In the present study, the complex between interleukin-19 (IL-19) and its physiological receptor—the interleukin-20 receptor α-chain (IL-20R1)—was modeled.

The most prominent feature of this complex is an extended binding interface formed by a long loop of IL-20R1 and a bulge region of IL-19. The two regions exhibit complementary charges and have no structural counterparts in the IL-10/IL-10R1 complex but show some resemblance to the complex between interferon-γ (IFN-γ) and its receptor.

Sequence comparison of the three cytokines (IL-19, IL-20, IL-24) that bind the IL-20R1 reveals a considerable conservation of the length of the interacting loops. One residue suggested to play a key role in receptor binding specificity is a conserved glutamate. The binding interface of IL-20R1 is rich in aromatic residues while the interfaces of its cytokine ligands are mainly formed by more flexible aliphatic amino acids. This structural feature might play an important role for the specific recognition of a single receptor chain by three different cytokines.

Keywords Interleukin · Receptor · Complex structure

Introduction

Cytokines are a group of proteins that play an important role in the immune system. A well-studied member is interleukin-10 (IL-10), a pleiotropic cytokine that has inhibitory and also stimulatory effects on various types of blood cells. Its primary function is to suppress immune response by blocking the synthesis of proinflammatory cytokines in T cells, monocytes, and macrophages and by inhibiting expression of cell-surface proteins involved in antigen presentation and costimulation. To date ten viral and five cellular homologs sharing 20–83% sequence identity to human IL-10 (hIL-10) are known. [1, 2, 3]

Cytokines of the IL-10 family exert their biological function by binding to a heterodimeric transmembrane receptor. This initiates a signaling pathway that involves activation of Jak kinases and the phosphorylation of signal transducer and activator of transcription (STAT) factors, which induce γ-activated sequence (GAS)-dependent or otherwise STAT-dependent transcription, for example induction of the gene encoding the suppressor of cytokine signaling 3 (SOCS-3). [4]

The receptors for the IL-10 family of cytokines belong to the cytokine receptor family type 2 (CRF-2). Their active form always consists of a long receptor chain (α- or R1-chain) that directly binds to the cytokine and is the major signaling component and of a smaller additional transmembrane subunit (β- or R2-chain). Characterization of the binding specificity of hIL-10 and its cellular paralogs (IL-19, IL-20, IL-22, and IL-24) revealed that IL-10 and IL-22 bind exclusively to the IL-10R1 and IL-22R α-chains, respectively. [5, 6, 7] In contrast, IL-19, IL-20 and IL-24 recognize the same α-chain (IL-20R1) in their receptor complexes. IL-20 and IL-24 but not IL-19 are also able to form an alternative signaling complex including the IL-22R α-chain. [8, 9] This complex pattern of receptor binding specificity together with tissue-specific expression of cytokines and receptors is considered to be critical for ensuring an efficient immune defense. [2]

The three-dimensional structures available for human IL-10, [10, 11] IL-19, [12] IL-22 [13] and the viral IL-10 homologs from Epstein–Barr virus (EBV) [14] and human cytomegalovirus [15] revealed that all these cytokines exhibit a very similar topology on the level of tertiary structure consisting of a six-helix bundle, but differ in their quaternary structure. Similar to interferon-γ

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(IFN-γ), [16] IL-10 and its viral homologs form intertwined dimers, while IL-19 and IL-22 are monomeric.

Information about the mode of receptor binding was obtained from the crystal structures of hIL-10 and hCMV-IL10 in complex with IL-10R1. [15, 17] These structures gave valuable information about the overall geometry of IL-10/IL-10R1 complexes—the receptor binding specificity in the IL-10 family, however, remains poorly understood since crystal structures containing other cytokine receptor α-chains have not been determined yet. For IL-22, the question of receptor binding specificity was addressed by building a three-dimensional model of the IL-22/IL-22R complex. Comparison to the crystal structure of the IL-10/IL-10R1 complex suggests that two receptor residues are largely responsible for the marked differences in ligand affinity observed for the two cytokines. [18] In contrast, no information is yet available how specific recognition of the IL-20R1 is achieved.

For that reason, we performed molecular modeling of an IL-19/IL-20R1 complex starting from the known IL-19 structure and the complex geometry of the IL-10/IL-10R1 complex. For the modeling of IL-20R1, sequence and structural information was included from a structure-based multiple sequence alignment generated for the CRF-2 family. The resulting model of the complex reveals several novel structural features that offer an explanation for the IL-20R1 binding specificity observed within the IL-10 family of cytokines.

Materials and methods

Sequence search and alignment

The existing alignment of the IL-10 family [1] was adjusted based on structural information that became available recently for hCMV-IL-10, [15] IL-19 [12] and IL-22 [13] using the DALI algorithm. [19]

Suitable templates for modeling the IL-20R1 chain were obtained from PSI-Blast [20] searches using the FoldPrediction(3D)-server (http://dove.embl-heidelberg.de/3D/). [21] This server is dedicated to finding homologous PDB sequences to a given query sequence. It uses a version of pdb that includes all the PDB entries. Sequences are compared to this database with PSI-BLAST, using an e-value cutoff of 0.001, and a maximum of five iterations. The accuracy of the fold prediction is estimated to be above 98%. [21]

In order to facilitate the identification of relevant hits, the length of the query sequence was restricted to the IL-20R1 extracellular region containing the cytokine binding interface. The boundaries of the extracellular region were obtained from searches using the programs SignalP [22] and HMMTOP, [23] giving information about the N-terminal signal sequence and the location of the transmembrane region, respectively. All hits obtained from the Blast search were subsequently confirmed by Pfam [24] and Dali [19] searches to exhibit the same domain organization and folding topology. In order to minimize misalignment due to low sequence similarity within the protein family, structural information was used to adjust the multiple sequence alignment whenever available.

Molecular modeling

Homology modeling of the IL-19/IL-20R1 complex was performed on the basis of the geometry of the IL-10/IL-10R1 complex in a two-step procedure. Firstly, the location of IL-19 in the complex was generated by a best-fit superposition of IL-19 on IL-10 based on the results of a Dali search, resulting in a (non-physiological) IL-19/IL-10R1 complex that can be modeled without any steric clashes.

This complex model served as a template for modeling IL-20R1 on the basis of IL-10R1 or on the basis of multiple templates from the group of IL-20R1 homologues of known three-dimensional structure. For modeling either Modeler 6.2 [25] or SwissModel [26] was used.

Modeling of loops was either performed by a direct alignment to sequences of known 3D structure or by using the strategy by Fiser et al., [27, 28] which relies on the satisfaction of spatial restraints rather than using templates of known 3D-structure (http://salilab.org/modloop).

The visualization of the structures was performed with Sybyl 6.9 (Tripos Inc.) and Rasmol. [29] The quality of the models was verified by Errat II [30] and Procheck. [31] Intermolecular contacts between receptor and ligand were analyzed with Ligplot [32] using a distance cutoff of 6 Å in order to take into account the limits of accuracy in modeling.

Results and discussion

Sequence alignment of the IL-10 cytokine family

The crystal structures that became available recently for hCMV IL-10, [15] IL-19, [12] and IL-22 [13] allowed the inclusion of additional structural information into the sequence alignment, thus making the alignment of sequences of low identity (~30%) much more reliable. Changes to a previous alignment [1] based on sequence information for these proteins alone are mainly located in the loops connecting the helices that are only moderately conserved in length and sequence (Fig. 1).

In principle, it should be possible to use the information present in this sequence alignment together with the structural data from the IL-10/IL-10R1 complex (Fig. 2a) to obtain first information about those residues that might play a role for receptor binding specificity. In order to identify residues important for IL-20R1 recognition, we searched for polar residues in the crystal structure of IL-19 that are conserved within the subgroup of IL-20R1 binding cytokines (IL-19, IL-20, IL-24) but not within the entire family of IL-10 related cytokines. Due to the low overall sequence identity of ~30% between IL-19, IL-20, and IL-24, only few such candidate residues could be identified. These were either located outside the receptor binding interface known from the IL-10/IL-10R1 complex (E57, Q60, K86) or buried in the interior of the protein (W164), thus giving little information about their potential role in receptor binding.

Structure comparison of IL-10 and IL-19

In order to obtain further information about structural features responsible for IL-20R1 binding specificity, the structure of the IL-19/IL-20R1 complex was modeled. IL-19 was chosen as representative of the IL-20R1 binding cytokines because the crystal structure of this protein is available, [12] thus facilitating model generation.

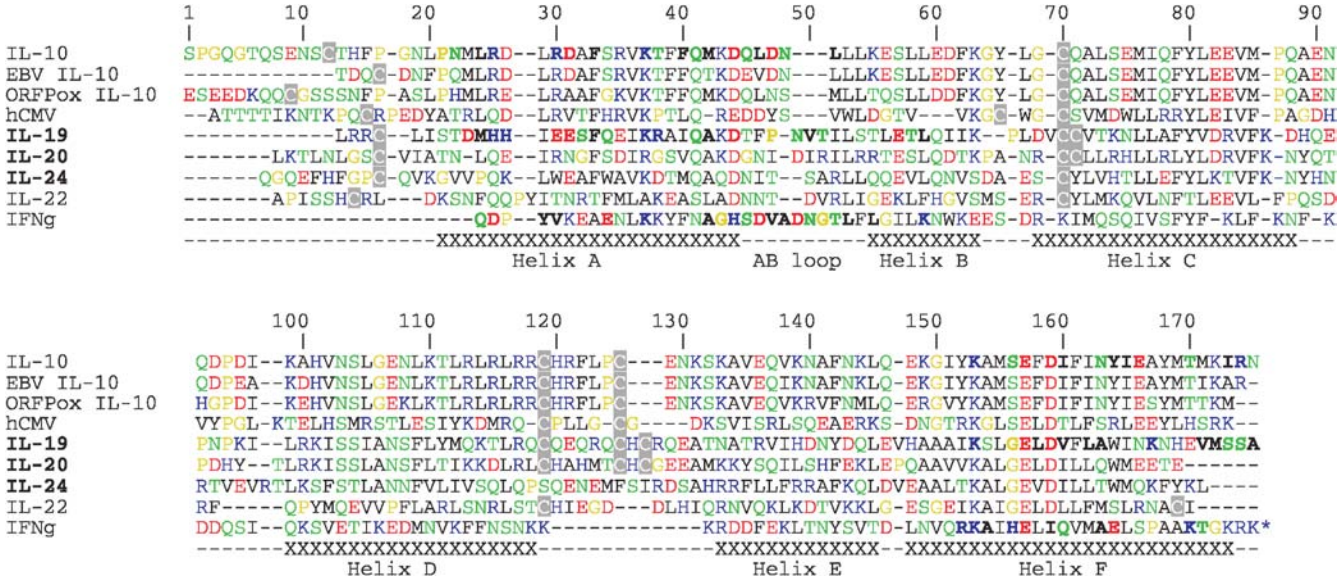

Fig. 1 Multiple Sequence alignment of viral and human members of interleukin 10 (IL-10) cytokine family. The viral sequence homologs encoded by Epstein–Barr virus (EBV), and human cytomegalovirus (hCMV) and the paralogous human cellular cytokines IL-19, IL-20, IL-24, and IL-22 have been aligned using the T-Coffee program. [35] The names of those proteins for which structural information is available are given in *bold* and their alignment was adjusted based on the three-dimensional structure using the DALI algorithm. The leader sequences have been removed based on their prediction using the SignalP program. [22] In addition, the sequence of human interferon (IFN-γ) is included, which is a close structural homolog of IL-10. The sequence truncation of IFN-γ at the C-terminus is indicated by an asterisk (*). Amino acids are colored according to the following scheme: *black*=hydrophobic (A, F, I, L, M, V, W, Y); *green*=polar (N, Q, S, T); *blue*=basic (R, K, H); *red*=acidic (D, E); *yellow*=special structural properties (C, G, P). Conserved cysteines that are known to form disulfide bonds are highlighted by *gray* boxes and the location and length of the helices A–F is indicated based on the IL-10 structure. Residues marked in *bold* are part of the receptor binding interface as deduced by LIGPLOT [32] analysis using a 6-Å cutoff criterion. Analysis was performed for the crystal structures of the IL-10/IL-10R1 [17] and the IFN-γ/receptor [34] complexes as well as for the model of the IL-19/IL-20R1 complex

Modeling was performed in a two-step strategy. First, a model of the IL-19/IL-10R1 complex was generated on the basis of the IL-10/IL-10R1 complex structure. Second, the structure of IL-20R1 in the complex was modeled resulting in the physiological IL-19/IL-20R1 complex.

Despite the relatively low overall sequence identity of 21%, IL-19 and IL-10 share the same folding topology, consisting of a six-helix bundle, and IL-19 can readily be superimposed on one domain of the intertwined IL-10 dimer (Fig. 2b, c). Interestingly, those regions known from the IL-10 complex structure [17] to be involved in receptor binding (Helices A and F) belong to those parts of the structure that exhibit the highest degree of structural conservation between IL-10 and IL-19 while the flanking regions (e.g. helix B) are less well conserved (Fig. 2d, e). Superimposition of IL-10 and IL-19 results in a low RMSD of 0.76 Å for the C_α atoms of the IL-10 receptor binding region. Therefore, an IL-19/IL-10R1 model was generated by replacing IL-10 by IL-19 in the IL10/IL10R1 complex structure, resulting in a complex without steric clashes. This modeling strategy does not account for an induced fit but appears to be justified by the observation that IL-10 undergoes only very subtle structural changes upon complex formation. [17]

Modeling of the IL-20R1 receptor chain

Subsequent modeling of the extracellular region of IL-20R1 in the complex proved to be more difficult, since no structural information is yet available for this receptor chain. Therefore, homology modeling was performed for IL-20R1.

Suitable homologs of known 3D structure were identified from a sequence search against the PDB database using the iterative strategy available at the FoldPrediction(3D) server. [21] The iterative search terminated after three rounds and listed a total of eight hits that cover the entire length of the query sequence and share a sequence identity of 21-24% to IL-20R1. These sequences originate from IL-10R1, human tissue factor and the IFN-α and IFN-γ receptors (PDB codes: 1FG9, 1FYH, 1AHW, 1FAK, 2HFT, 1A21, 1J7V, 1N6U). According to the Dali classification, all hits share the same fold ("tissue factor fold"; Dali Fold Nr. 851), consisting of two fibronectin domains, and exhibit significant pairwise Dali Z-scores >11.2. This list was further extended by human growth hormone receptor (hGHR; pdb code: 1KF9), which represents an additional structural homolog belonging to the "tissue factor fold" family. The resulting multiple sequence alignment, based on structural information whenever available, is shown in Fig. 3. The protein family shows an average pairwise sequence identity of 20–30%

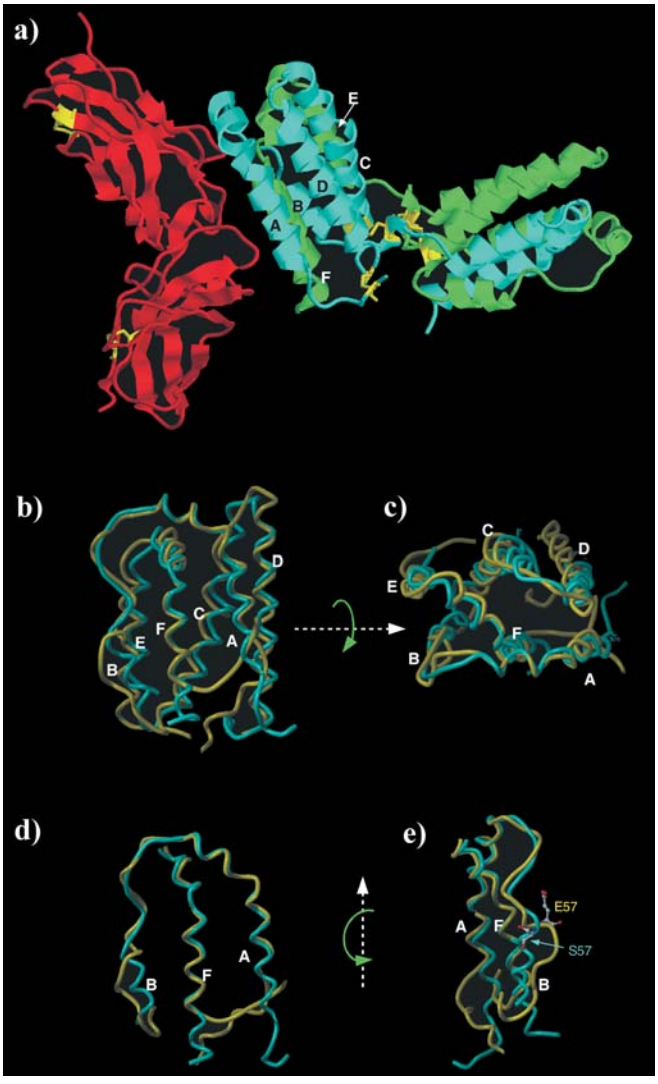

Fig. 2 Comparison of the IL-10 and IL-19 structures. **a** Crystal structure of the IL-10/IL-10R1 complex. [17] The subunits of the intertwined IL-10 dimer are colored in *cyan* and *green*, respectively. The six helices of one domain of IL-10 are labeled. The IL-10R1 is shown in *red* and cysteines that form disulfide bonds are shown in *yellow* as stick representation. **b** Backbone overlay of the IL-19 crystal structure (*yellow*) onto one domain of IL-10 (*blue*). The six conserved helices are labeled. **c** Same overlay as in **b** but rotated by 90° around the horizontal axis. **d** Backbone overlay of the receptor binding regions of IL-10 and IL-19. The two helices A and F can be superimposed with an RMSD of 0.76 Å, while the adjacent helix B shows larger structural differences. **e** Same overlay as in **d** but rotated by 90° around the vertical axis. Residues S57 and E57 that are located in helix B of IL-10 and IL-19, respectively, are shown in ball-and-stick representation

and mainly differs in the length of the loops connecting the β-strands.

Typically, there are two conserved disulfide bonds in the receptors of known structure present at identical positions (C61–C72; C226–C249). This characteristic pattern and spacing of cysteines is also observed in IL-20R1, rendering an identical disulfide bonding pattern highly likely. In addition to the human growth hormone receptor, which lacks the second disulfide bond, the second notable exception is the IL-10R1, in which the spacing and position of the first two cysteines differ from those of the remaining members of the family.

Based on the multiple sequence alignment, several modeling strategies were tested in order to obtain the IL-20R1 model. For the overall backbone topology, two models were generated, one on the basis of the pairwise IL-10R1/IL-20R1 alignment (Fig. 3) and one on the basis of multiple templates including the structural information from all receptor chains listed in Fig. 3. In both models, the conserved cysteines are in disulfide binding distance, suggesting that the overall alignment is correct. According to the Procheck and Errat analysis, the model based solely on the IL-10R1 structure shows the better covalent and noncovalent structural parameters.

Due to the differences in sequence length, however, loop L2 exhibited a poor geometry in both models. Therefore, the loop from residue Y49–L56 of the IL-20R1 was modeled according to the strategy by Fiser et al. [27, 28] that relies on the satisfaction of spatial restraints rather than using templates of known 3D structure. As noted previously for other proteins, [27] this strategy resulted in an improvement of the loop geometry of IL-20R1. Therefore, the final model was generated on the basis of the IL-10R1/IL-20R1 alignment shown in Fig. 3 supplemented by a separate prediction for loop L2.

Structural analysis of the IL-19/IL-20R1 complex

The resulting model (Fig. 4a) was analyzed to obtain information about the properties of the complex interface and the nature of the intermolecular contacts. In order to take into account the limited accuracy of modeled in comparison to experimentally determined structures, all residues closer than 6 Å to the interaction partner were considered as contact region (Figs. 3 and 4b).

One interesting feature of the IL-20R1 interface (Fig. 4b) is the high number of solvent-exposed aromatic residues (F47, Y49, W55, Y87, F117, Y118, F120, F232) compared to IL-10R1 (Fig. 3). Such aromatic residues generally form rigid scaffolds with distinct structural properties for the recognition of interaction partners. [33] In contrast, aromatic residues are almost completely absent from the interaction surface of IL-19 (Figs. 1 and 4b) and the hydrophobic interactions are mainly formed by aliphatic side chains. The fact that this structural feature is not only present in IL-19 but also in IL-20 and IL-24 suggests that an increased structural plasticity of aliphatic compared to aromatic side chains might play a general role for the recognition of the single IL-20R1 receptor chain by different cytokine ligands.

In addition to hydrophobic contacts, several clusters of interactions between polar residues have also been identified in the model of the complex (Fig. 4b). Some of them, e.g. that formed by D159 of IL-19 and R116 of IL-20R1 (Fig. 4b), are formed in an identical fashion in the IL-10/IL-10R1 complex. Because of the strict conservation of the corresponding residues, in the IL-10 family

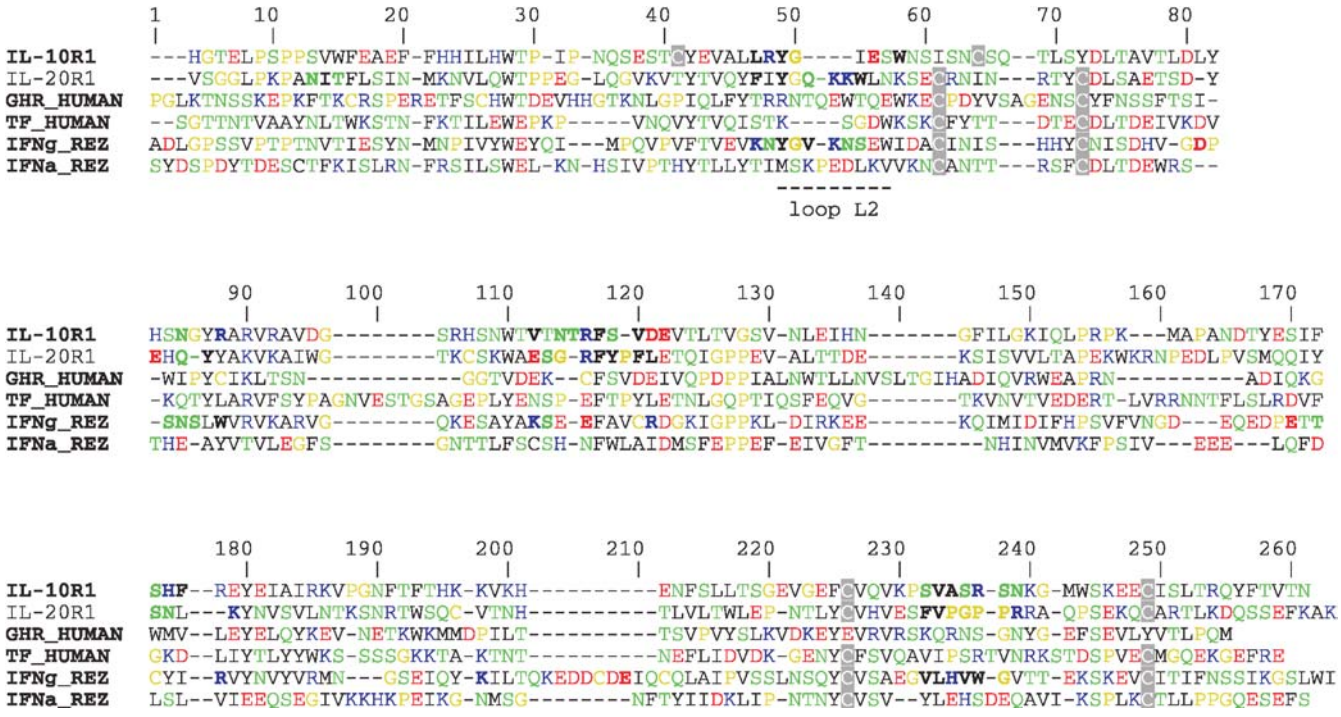

Fig. 3 Multiple Sequence alignment of receptors of the "tissue factor" family, including IL-10R1, IL-20R1, tissue factor, and the receptors for human growth hormone IFN-γ, and IFN-α. The names of those proteins for which structural information is available are given in *bold* and their sequences were aligned based on the three-dimensional structure using the DALI algorithm. Alignment of IL-20R1 for which no structural information is available was performed according to the multiple sequence alignment available from the Pfam database. Amino acids are colored according to the following scheme: *black*=hydrophobic (A, F, I, L, M, V, W, Y); *green*=polar (N, Q, S, T); *blue*=basic (R, K, H); *red*=acidic (D, E); *yellow*=special structural properties (C, G, P). Conserved cysteines that are known to form disulfide bonds are highlighted by *gray* boxes and the position of loop L2 is indicated. Residues marked in *bold* are part of the receptor binding interface as deduced by LIGPLOT [32] analysis using a 6-Å cutoff criterion. Analysis was performed for the crystal structures of the IL-10/IL-10R1 [17] and the IFN-γ/receptor [34] complexes as well as for the model of the IL-19/IL-20R1 complex

(Fig. 1) and the corresponding receptors (Fig. 3) they are expected to play a general role in cytokine receptor recognition. Others, e.g. between the C-terminal residues of IL-19 and the second domain of IL-20R1 (Fig. 4b), cannot be formed by IL-20 and IL-24 because of their shorter C-termini (Fig. 1). Therefore, these interacting regions are not expected to play a crucial role for specific recognition of IL-20R1.

With respect to specific recognition, another part of the interface formed between Loop L2 of IL-20R1 and the AB loop+helix B of IL-19 is of particular interest because these regions exhibit unique structural features not present in the IL-10/IL-10R1 complex. The loop L2 in IL-20R1 is significantly longer than the corresponding loop in IL-10R1 (Fig. 3) and contains an excess of positively charged amino acids.

The interacting residues of IL-19 are mainly polar in nature and are exposed by the AB loop and helix B of IL-19. In IL-19 and the other IL-20R1 binding cytokines, the AB loop is longer compared to IL-10 and the residues inserted around position 50 are part of the receptor binding interface (Fig. 1). Helix B of IL-10 is a regular α-helix, which is tightly packed against the core of the protein and does not form contacts with the IL-10R1. [17] In IL-19 helix B is much shorter and a 3_{10} helix followed by a loop is present instead (Fig. 2d). The corresponding structural element points away from the core in the IL-19 crystal structure (Fig. 2e) and is oriented towards the IL-20R1 interface in the model of the complex (Figs. 4b and 5b).

Thus, the existence of a longer loop in IL-20R1 as well as the presence of a two-residue insertion in the AB loop and the differences of the conformation in helix B in IL-19 create a novel interaction surface in the IL-19/IL-20R1 complex not present in the IL-10/IL-10R1 complex (Fig. 5a, b). Interestingly, a similar extended interaction surface formed between the corresponding parts of the interaction partners is present in the structure of the IFN-γ/receptor complex (Fig. 5c). Although the information about the geometry of the IFN-γ/receptor complex [16, 34] was not used in any stage of the modeling, comparison of the contacts between the AB loop and loop L2 (Figs. 1 and 3) reveals that the IL-19/IL-20R1 contacts are more similar to those present in the IFN-γ/receptor complex than to those present in the IL-10/IL-10R1 complex.

The limited accuracy of the present model does not allow the analysis of the contacts in the IL-19/IL-20R1 complex on an atomic level. One residue, however, that might play a key role for specific receptor recognition in

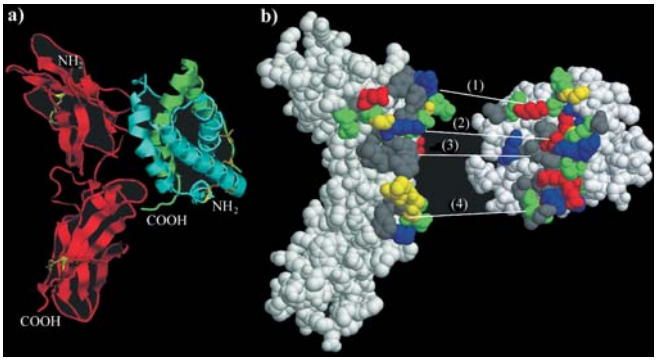

Fig. 4 Model of the IL-19/IL-20R1 complex. **a** Backbone presentation of the IL-19/IL-20R1 complex. For clarity, the same orientation and color coding were used as for the IL-10/IL-10R1 complex shown in Fig. 2: Helices A–D and E–F of IL-19 are shown in *cyan* and *green*, respectively and IL-20R1 is shown in *red*. Cysteines that form disulfide bonds are shown in *yellow* as stick representation and the N- and C- termini are indicated. **b** Surface representation of IL-20R1 and IL-19. IL-20R1 is shown in a similar orientation as in Fig. 4a while IL-19 was rotated by approximately 90° around the vertical axis in order to allow a view on the complex interface. Residues involved in intermolecular contacts are colored according to the following scheme: *gray*=hydrophobic (A, F, I, L, M, V, W, Y); *green*=polar (N, Q, S, T); *blue*=basic (R, K, H); *red*=acidic (D, E); *yellow*=special structural properties (C, G, P). Lines connect clusters of interacting residues between IL-19 and IL-20R1: (1) N49, E57/Q51, K54; (2) D159/R116; (3) V160, L162, A163/Y118, F120; (4) M172-A175/V233-P238

IL-19 is E57. This residue is involved in receptor contacts in the model and is exclusively present in the three cytokines that bind IL-20R1 (Fig. 1). This residue is located at an exposed position in IL-19 that has no structural equivalent in IL-10 (Fig. 2e) due to the differences in the orientation of helix B.

Interestingly, in the IL-22 crystal structure, [13] the corresponding region adopts a loop structure similar to that observed in IL-19 (data not shown). At position 57, however, a lysine is present instead of a glutamate (Fig. 1), giving a likely explanation for the differences in receptor binding specificity observed between the two cytokines. The latter observation adds further support to the assumption that the corresponding sequence regions play an important role for recognition in cytokine–receptor complexes.

In summary, molecular modeling suggests that structural differences of the AB loop and helix B in conjunction with surface and charge complementarity of receptor loop L2 play a role in differences in receptor binding specificities observed in the IL-10 cytokine family. The present computational study provides the basis for further experimental investigations in order to prove or disprove the role of particular residues for receptor binding specificity. In addition, this molecular description of IL-20R1 binding should be helpful for the study and design of drugs that specifically target the IL-20R1 or a subgroup of cytokines from the IL-10 family.

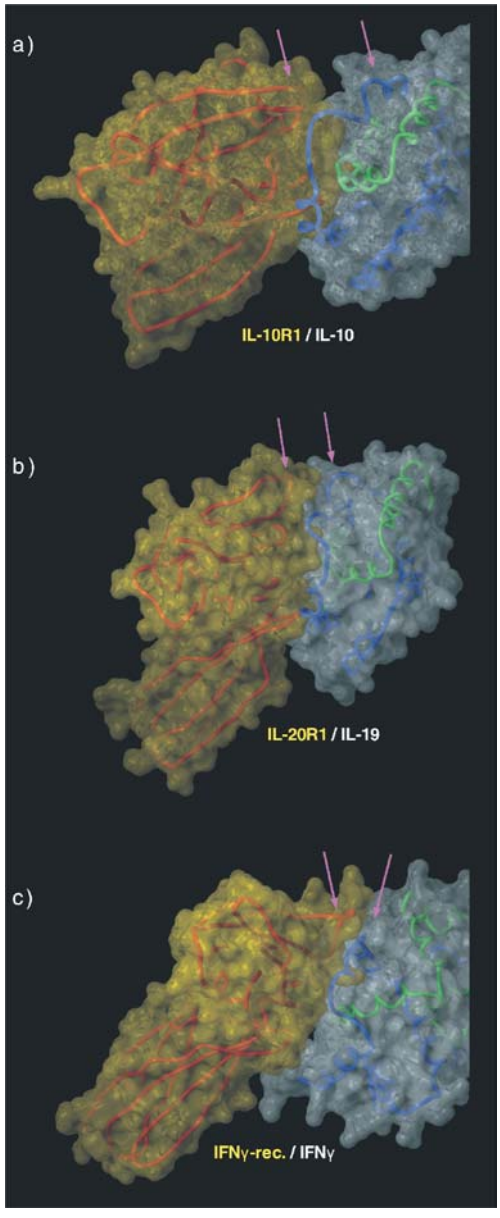

Fig. 5 Comparison of the ligand/receptor interfaces in the **a** IL-10/IL-10R1, **b** IL-19/IL-20R1 and **c** IFN-γ/receptor complexes. The translucent Connolly surfaces of the receptor and the ligand are shown in *yellow* and *white*, respectively. The backbone of the receptor and ligand are highlighted by a *red* and *blue/green* tube, respectively. For clarity, only one domain of the intertwined dimes is shown for IL-10 and IFN-γ. Arrows denote the location of helix B and the corresponding structural elements in IL-19 and IFN-γ as well as the location of receptor loop L2. As evident from **b** and **c** an extended interaction surface is created in the IL-19/IL-20R1 and IFN-γ/receptor complexes by the interaction of this structural element with a long loop of the respective receptor

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