

# The interleukin-10 family of cytokines

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A family of interleukin-10 (IL-10)-related cytokines has emerged, comprising a series of herpesviral and poxviral members and several cellular sequence paralogs, including IL-19, IL-20, IL-22 [IL-10-related T-cell-derived inducible factor (IL-TIF)], IL-24 [melanoma differentiation-associated antigen 7 (MDA-7)] and IL-26 (AK155). Although the predicted helical structure of these homodimeric molecules is conserved, certain receptor-binding residues are variable and define the interaction with specific heterodimers of different type-2 cytokine receptors. This leads, through the activation of signal transducer and activator of transcription (STAT) factors, to diverse biological effects. For example, whereas IL-10 is a well-studied pleiotropic immunosuppressive and immunostimulatory cytokine, IL-22/IL-TIF mediates acute-phase response signals in hepatocytes and IL-20 induces the hyperproliferation of keratinocytes, which has been proposed as a pathogenic mechanism of psoriasis.

Interleukin-10 (IL-10) was discovered initially as an inhibitory factor for the production of T helper 1 (Th1) cytokines. Subsequently, pleiotropic inhibitory and stimulatory effects on various types of blood cells were described for IL-10, including its role as a survival and differentiation factor for B cells. IL-10, which is produced by activated monocytes and T cells, as well as other cell types, such as keratinocytes, appears to be a crucial factor for at least some forms of peripheral tolerance and a major suppressor of the immune response and inflammation. The inhibitory function of IL-10 is mediated by the induction of regulatory T cells (reviewed in Ref. [1]).

The first suggestion of an IL-10 family was the discovery of a functional sequence homolog of IL-10 in the genome of the human  $\gamma_1$ -herpesvirus/lymphocryptovirus Epstein-Barr virus (EBV) [2,3]. Because infection with EBV leads to the growth transformation of primary human B cells, this discovery suggested an autocrine transformation mechanism through secreted EBV IL-10. Indeed, it is difficult to distinguish the biological activities of cellular and EBV-derived IL-10 molecules [4]. However, cellular IL-10 was shown to be functionally dominant over viral IL-10 in EBV-transformed human B cells [5]. Moreover, the EBV gene BCRF1, which encodes viral IL-10, was shown to be dispensable for B-cell transformation *in vitro* [6]. The genomes of lymphocryptoviruses of other Old World primate species seem to encode similar proteins, as shown for herpesvirus papio (HVP) of baboons (GenBank™ accession number AAF23949). Additional highly conserved homologs of IL-10 (70–80% identity) were discovered in the genomes of the  $\gamma_2$ -herpesvirus/rhadinovirus equine herpesvirus type 2 (EHV-2) [7] and the ovine Orf parapoxvirus [8,9]. Recently,

Table 1. Viral members of the interleukin-10 family

Viral homolog	Source	Identity to human IL-10 (%)
EBV IL-10	Epstein-Barr virus	83
HVP IL-10	Herpesvirus papio	78
EHV-2 IL-10	Equine herpesvirus 2	76
HCMV IL-10	Human cytomegalovirus	27
SCMV IL-10	Simian cytomegalovirus	29
Orf IL-10	Ovine Orf parapoxvirus	73
YLDV IL-10	Yaba-like disease poxvirus	30

more distantly related molecules (<30% identity) have been discovered in the genomes of  $\beta$ -herpesviruses, namely human and simian cytomegaloviruses (HCMV and SCMV) [10,11], and the monkey yatapoxvirus Yaba-like disease virus (YLDV) [12]. Thus, the family of viral homologs of IL-10 comprises presently seven members, all from large DNA viruses, specifically herpesviruses and poxviruses (Table 1 and Fig. 1). The viral members of the IL-10 family, as shown for EBV, CMV and Orf parapoxvirus, seem to share the specific receptor and thus, major functional properties of cellular IL-10 [4,8–10].

Three cellular paralogs of IL-10 – melanoma differentiation-associated antigen 7 (MDA-7; renamed as IL-24), IL-10-related T-cell-derived inducible factor (IL-TIF; renamed as IL-22) and AK155 (renamed as IL-26) – were discovered by subtractive cDNA hybridization, owing to their specific overexpression in defined cell types. IL-24/MDA-7 (synonyms C49a, FISP, Mob-5 and ST16) is expressed by differentiated melanoma cells and Th2 lymphocytes, and was described to have anti-tumor activity [13–18]. IL-22/IL-TIF is produced by CD4<sup>+</sup> T cells; its expression can be induced by IL-9 in murine thymic lymphoma cells, and it mediates acute-phase response signals in hepatocytes [19–21]. Transcripts of IL-26/AK155 were found at high levels in human T cells that had been growth-transformed in culture by herpesvirus saimiri, a  $\gamma_2$ -herpesvirus/rhadinovirus of the neotropical primate species *Saimiri sciureus* (squirrel monkey) [22,23]. The additional paralogs, IL-19 and IL-20, were discovered when sequence data from genomic and cDNA libraries became available in private databases. Together with the gene encoding IL-24/MDA-7, the genes encoding IL-19 and IL-20 cluster into the genomic IL-10 region on human chromosome 1 [24,25]. By contrast, the human genes encoding IL-22/IL-TIF and IL-26/AK155 are situated

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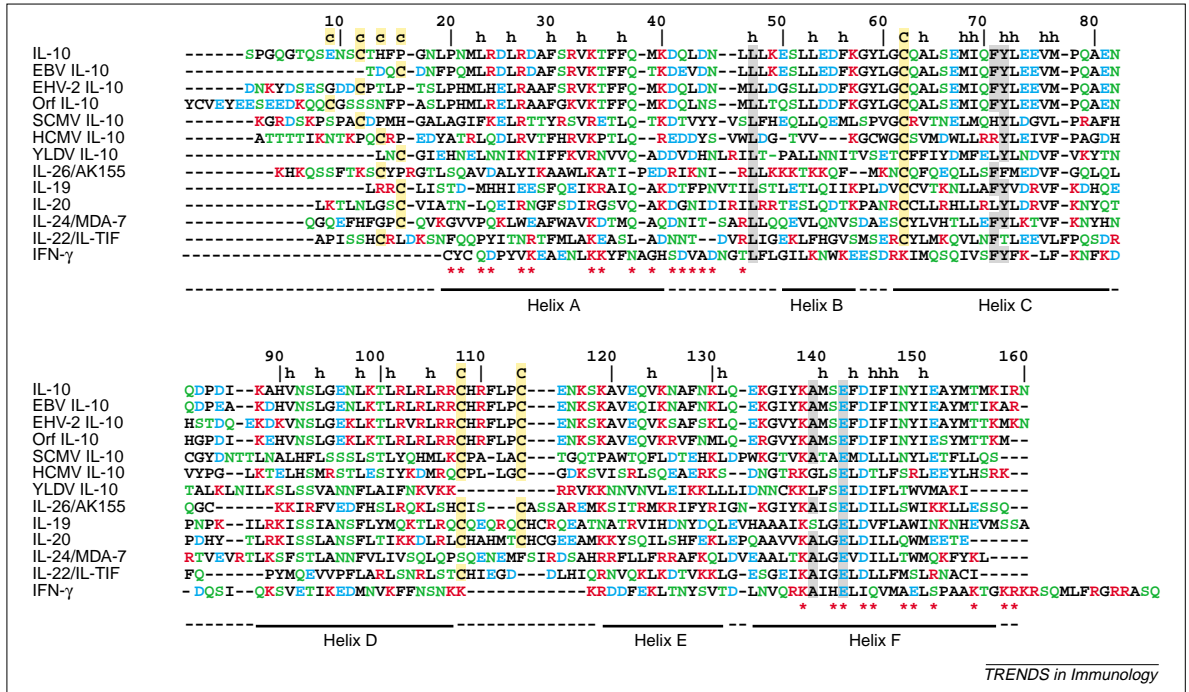


Fig. 1. Multiple sequence alignment of viral and human members of the interleukin-10 (IL-10) cytokine family. The viral sequence homologs encoded by Epstein-Barr virus (EBV), equine herpesvirus 2 (EHV-2), ovine Orf parapoxvirus, simian cytomegalovirus (SCMV; from the rhesus monkey), human cytomegalovirus (HCMV; strain Towne) and the simian Yaba-like disease yatapoxvirus (YLDV) are aligned in comparison with human IL-10. In addition, the paralogous human cellular cytokines IL-26/AK155, IL-19, IL-20, IL-24/melanoma differentiation-associated antigen 7 (MDA-7) and IL-22/IL-10-related T-cell-derived inducible factor (IL-TIF) have been aligned. For comparison, the sequence of human interferon  $\gamma$  (IFN- $\gamma$ ) is shown, which is the closest human IL-10 homolog of known three-dimensional structure. The leader sequences have been removed based on their prediction using the SignalP program [58]. The amino acid residues are numbered according to the mature form of IL-10. Residues are colored according to their physico-chemical properties: positively charged (K and R) in red; negatively charged (D and E) in blue; polar (N, Q, S and T) in green; and nonpolar or aromatic (A, F, G, H, I, L, M, F, V, W and Y) or cysteine (C) in black. The positions of the structurally relevant cysteine residues are indicated by yellow boxes and additionally marked by the upper-case letter 'C' in the headline; N-terminal to helix A, a lower-case letter 'c' marks the varying position of the first cysteine. The length of the helices A to F in the IL-10 structure is indicated below the alignment. Hydrophobic residues that are conserved in at least 90% of the IL-10 family members are marked with the letter 'h' in the headline. In the amphipathic helices A, B and D, two to three residues are interspersed between the conserved hydrophobic residues, whereas helices C and F contain more closely spaced hydrophobic residues, involved in hydrophobic contacts with several other helices. Red asterisks mark those residues forming the receptor-binding interface in the IL-10/IL-10R1 complex [32]. The structurally relevant and conserved residues L47, F71, Y72, A139 and E142 are shaded in gray. The alignment was generated using the T-COFFEE program [59] and modified subsequently using structural information about the location of the helices and cysteine residues forming the disulfide bonds. The alignment between IL-10 and IFN- $\gamma$  was performed on the basis of their three-dimensional structures using the DALI algorithm [60].

in close vicinity to the gene encoding interferon  $\gamma$  (IFN- $\gamma$ ) on chromosome 12 [22,26]. IL-19 is transcribed in activated monocytes [25]. Although it remains unclear which cell types produce IL-20, the skin has been suggested as its main target tissue. The overexpression of IL-20 in transgenic mice induced

lesions similar to those observed in psoriasis [24]. Thus, presently, five cellular molecules with structural similarity, but distinct expression patterns and diverse functions, have joined the IL-10 family (Tables 2,3 and Fig. 1).

**Structural features of the IL-10 family**

Three-dimensional structures are known only for human IL-10 [27–29] and its viral homolog from EBV [30]. Both molecules form V-shaped dimers (Fig. 2a). Each arm of the V-shaped dimer consists of six  $\alpha$ -helices, four originating from one subunit (A–D) and two from the other subunit (E' and F'). Four of the  $\alpha$ -helices (A, C, D and F') form a typical bundle [27] that is observed also in all other helical cytokines, such as IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Figs 1,2a). The C-terminal two helices of one molecule (E and F) penetrate into the N-terminal four-helix subunit (A'–D') of the dimerization partner (Fig. 2a). The overall topology of the helices in IL-10 and their quarternary arrangement resemble strongly that of IFN- $\gamma$  [31] and demonstrate the close relationship between the two cytokines.

The various IL-10-related cytokines have sequence identities of 20–83% with human IL-10 (Tables 1,2

**Table 2. Cellular members of the interleukin-10 family<sup>a</sup>**

Human cellular paralog	Chromosomal localization	Identity to human IL-10 (%)
IL-10	1q32	100
IL-19	1q32	20
IL-20	1q32	28
IL-24/MDA-7	1q32	23
IL-22/IL-TIF	12q15	26
IL-26/AK155	12q15	27

<sup>a</sup>Abbreviations: IL-TIF, interleukin-10-related T-cell-derived inducible factor; MDA-7, melanoma differentiation-associated antigen 7.

**Table 3. Expression patterns of interleukin-10 family members in human lymphocytes<sup>a</sup>**

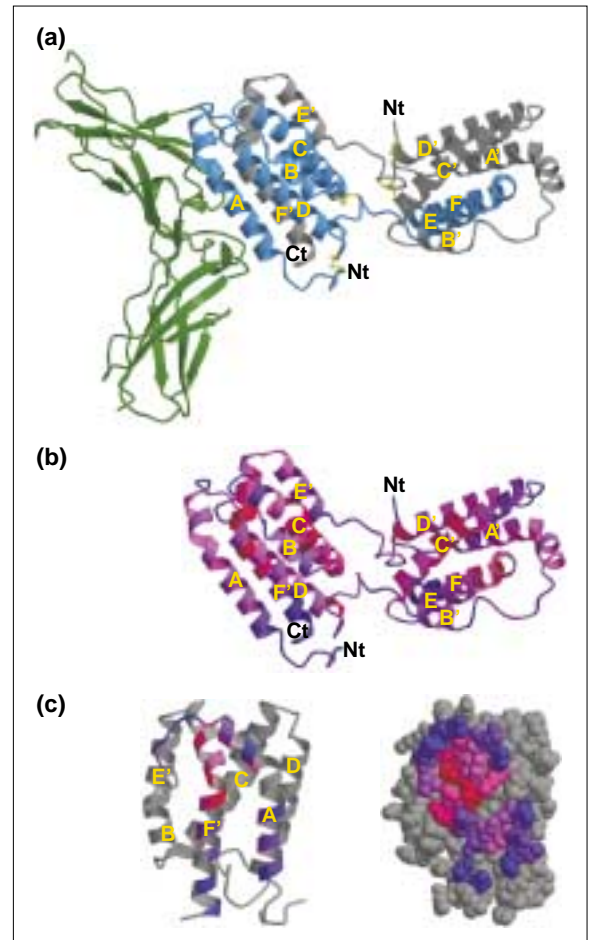
Cell type	Expression level <sup>b</sup>				
	IL-10	IL-19	IL-24/ MDA-7	IL-26/ AK155	IL-22/ IL-TIF
T lymphoblasts	++	+	+	+++	+++
Phorbol-ester-stimulated T lymphoblasts	++	+	+	+++	+++
Herpesvirus-saimiri-transformed T cells	+++	-	-	+++	+++
T-leukemia cells	++	-	-	+++	+
HTLV-1-transformed T-cell lines	+++	+++	+	++	-
KSHV <sup>+</sup> BCBL-1 cell lines	+++	+++	+	+	+
B-cell lines	+++	++	+	-	-
EBV lymphoblastoid cell line	+++	+++	+	-	-
Fresh blood cells	++	+++	++	++	+

<sup>a</sup>Abbreviations: BCBL-1, body-cavity-based lymphoma cell line 1; EBV, Epstein-Barr virus; HTLV-1, human T-cell leukemia virus type 1; IL-TIF, interleukin-10-related T-cell-derived inducible factor; KSHV, Kaposi's-sarcoma-associated herpesvirus; MDA-7, melanoma differentiation-associated antigen 7.

<sup>b</sup>Expression levels were measured by semiquantitative RT-PCR and confirmed by Southern blot hybridization. Symbols: +++, strong signal; ++, weak signal; +, detectable only after hybridization; -, no signal detectable [22].

and Fig. 1). High sequence identities of ~80% are observed between IL-10 and the viral homologs from EBV, the closely related HVP of baboons, EHV-2 and the ovine Orf parapoxvirus. However, a low degree of conservation is observed between cellular IL-10 and the viral counterparts from CMV and YLDV. A similar low sequence homology of ~25% identity and 50% similarity is encountered with the human cellular paralogs IL-19, IL-20, IL-22/IL-TIF, IL-24/MDA-7 and IL-26/AK155. By contrast, the sequence identity between the human and murine cellular orthologs of IL-10 and IL-22/IL-TIF remains high (72% and 81%, respectively), demonstrating that the orthologs of different mammalian species share considerably more similarity in structural and functional terms than the different human IL-10 paralogs.

The degree of sequence conservation of the viral and cellular relatives of IL-10 suggests that all these molecules are structural homologs of IL-10 [32]. Moreover, the amphipathic pattern of hydrophobic and hydrophilic residues for the six helices is well conserved in all sequences (Fig. 1). Generally, the most highly conserved stretches of sequence are found in the C-terminal half of helix C and the N-terminal half of helix F, which forms a pronounced bend (Fig. 2b). Also, these two regions contain four (Phe71, Tyr72, Ala139 and Glu142) of the five amino acids (Leu47, Phe71, Tyr72, Ala139 and Glu142) reported to be key residues in stabilizing the structural core in IL-10 and IFN- $\gamma$  [29] (Fig. 1). These residues are conserved in most members of the IL-10 family, suggesting a similar helix arrangement. By contrast, the pattern and spacing of the four cysteines forming two intramolecular disulfide bonds in IL-10 – in a 1–3 and 2–4 fashion – is not conserved strictly in the family (Fig. 1), suggesting that the presence of disulfide bonds might not be absolutely necessary for maintaining the IL-10 fold. This is in agreement with the fact that IFN- $\gamma$  adopts the same fold, but has no disulfide bonds at all [27,29]. Regions



**Fig. 2. Structural features of interleukin-10 (IL-10) family proteins.** (a) Crystal structure of the IL-10/IL-10 receptor 1 (IL-10R1) complex [32]. The two interpenetrating dimer-forming subunits of IL-10 are colored in blue and gray. The cysteines forming the disulfide bonds are shown in yellow. The helices (A–F) are labeled, and the N-terminus (Nt) and C-terminus (Ct) are indicated. For clarity, only the IL-10R1 chain that binds to the left-hand half of the dimer is shown (in green). (b) Sequence conservation in the IL-10 family. The degree of sequence conservation of the IL-10-related cytokines to IL-10 was mapped onto the three-dimensional structure model of IL-10. The color gradient ranges from red for highly conserved regions to blue for regions showing only low sequence conservation. Conservation values were calculated from the multiple sequence alignment shown in Figure 1 using the plotsimilarity subroutine of the GCG package (Genetics Computing Group, Madison, WI, USA) with a window size of five residues. (c) Conservation of the IL-10 residues forming the IL-10/IL-10R1 interface. In the ribbon (left) and space-filled (right) representations, residues forming the IL-10/IL-10R1 interface are colored from red for highly conserved amino acids to blue for residues showing only low sequence conservation in the family of IL-10-related cytokines [32]. Residues of IL-10 that are thought not to be involved in receptor interactions are shown in gray. The conservation was calculated from the multiple sequence alignment shown in Figure 1 using the plotsimilarity subroutine of the GCG package. The view is rotated by approximately 90° anticlockwise around the vertical axis compared with (a) and (b), and for clarity, only the left-hand half of the IL-10 dimer is shown.

exhibiting low sequence similarity within the IL-10 family include the N-terminal residues and the loops connecting the helices, particularly the C–D and D–E loops (Figs 1,2b).

The formation of a stable monomer would require helices E and F to fold back into the cleft created by

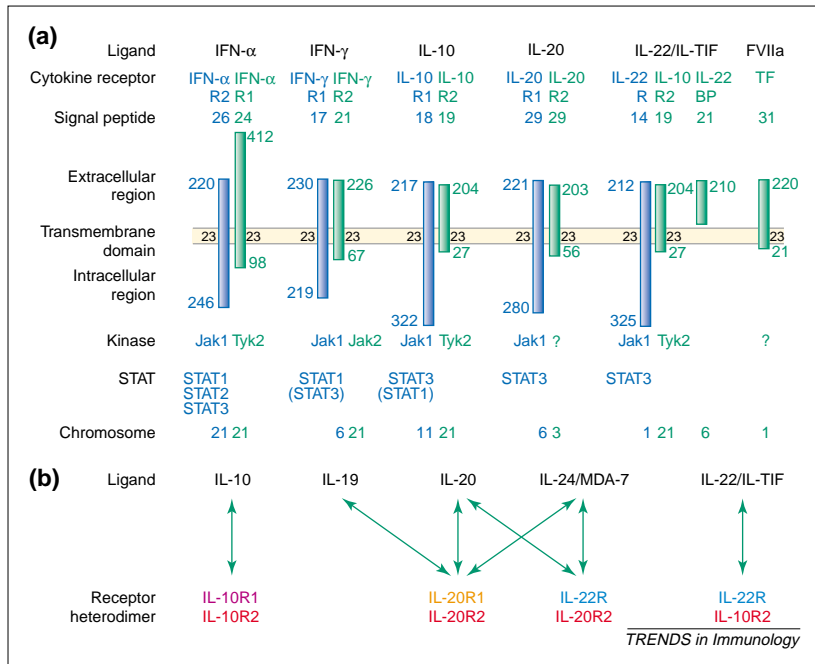


Fig. 3. Receptors for interleukin-10 (IL-10) family proteins. (a) Cytokine receptor family type-2 members are receptors for interferons (IFNs) and IL-10-related cytokines (modified from Ref. [36]). The lengths of the signal peptide, and extracellular, transmembrane and intracellular domains are given in amino acid numbers. (b) Ligand-receptor assignment. Recently, additional receptor dimers have been assigned to IL-19, IL-20 and IL-24/melanoma differentiation-associated antigen 7 (MDA-7) [43]. IL-19, IL-20 and IL-24/MDA-7 use the IL-20R1/IL-20R2 heterodimer. In addition, IL-20 and IL-24/MDA-7, but not IL-19, bind to IL-22R/IL-20R2 complexes. Abbreviations: BP, binding protein; FVIIa, blood coagulation factor VIIa; IL-TIF, IL-10-related T-cell-derived inducible factor; STAT, signal transducer and activator of transcription; TF, tissue factor.

helices A to D of the same chain, thus, occupying the position of helices E' and F' in the dimer (Fig. 2a). In modeling studies, a monomeric form of IL-10 could be created only by reduction of the Cys62–Cys114 disulfide bond or if the structure was otherwise seriously distorted [27,29]. Recently, a monomeric human IL-10 was designed by inserting six amino acids into the D–E loop, between residues 116 and 117. This engineered cytokine formed a stable  $\alpha$ -helical monomer, which closely resembled one domain of the dimeric wild-type IL-10 and was biologically active [33]. Notably, IL-22/IL-TIF lacks one cysteine (Cys114) in the loop connecting helices D and E (Fig. 1). Therefore, no steric constraint is placed on the conformation of the D–E loop by a disulfide bond, which might allow this cytokine to form stable monomers. Instead, a cysteine is present at the C-terminus of helix F (Cys154), suggesting that an alternative disulfide bond might be formed in IL-22/IL-TIF. The otherwise conserved cysteine (Cys114) is absent also from the D–E loop of IL-24/MDA-7 and YLDV IL-10, suggesting that these cytokines might form stable monomers also.

#### Receptor binding by IL-10

The active IL-10-receptor signaling complex contains two different receptor chains, IL-10R1 and IL-10R2, the latter also termed cytokine-receptor

family type-2 member 4 (CRF2-4) [34–36]. Complex formation occurs in two steps: initially, IL-10 binds with high affinity to the larger receptor chain, IL-10R1, and subsequently, IL-10R2 is bound with lower affinity. Recently, the crystal structure of the IL-10/IL-10R1 complex has been determined [32], revealing the molecular details of the interaction of IL-10 with its receptor (Fig. 2a). In this complex, the dimeric IL-10 molecule binds symmetrically to two soluble extracellular IL-10R1 chains, demonstrating an analogous topology to the IFN- $\gamma$ /IFN- $\gamma$ R1 complex [31,37]. Evidence for the existence of a larger complex, consisting of two IL-10 dimers and four soluble IL-10R1 chains, is provided by gel-filtration experiments [38] and crystallographic data [32]. Based on these observations, it was proposed that this complex is itself a model of the biologically active IL-10/IL-10R1/IL-10R2 complex, such that the IL-10R1 molecules mimic the low-affinity interaction of IL-10R2 with IL-10, suggesting that IL-10R1 and IL-10R2 recognize the same binding site on IL-10 [32].

Twenty-seven amino acids located in helix A, the A–B loop and in helix F' of IL-10 contact IL-10R1 (Fig. 2) [32]. In the three-dimensional structure, these residues are in close spatial proximity (Fig. 2c), thus providing a continuous receptor-binding surface. Three of these sequence positions are occupied exclusively by charged residues throughout the entire IL-10 family, and also in IFN- $\gamma$ . Whereas Lys138 and Glu142 are strictly conserved, either a lysine or an arginine can be present at position 34. In addition, there are six amino acids (Leu23, Arg27, Gln38, Asp41, Asp144 and Glu151) that are conserved significantly between human IL-10 and the viral IL-10 homologs, suggesting that these residues might be responsible for the conserved activities of these proteins. Color coding of the residues involved in receptor binding, according to their degree of conservation in the various IL-10 family members (Fig. 2c), reveals the presence of a central hot spot of conservation, formed by the highly conserved residues, surrounded by less-conserved contact residues [32].

#### The cytokine receptor family type 2

The cytokine receptor family type 2 (CRF2) comprises the various IFN receptor chains and the receptors for IL-10-related cytokines (Fig. 3a) [36]. As a prototype, the IL-10 receptor consists of a long chain (IL-10R1) [34], which is the major signaling component, and an additional membrane-spanning receptor chain (IL-10R2 or CRF2-4) [35] with a short intracellular segment. Similarly, all the other receptor heterodimers of this family combine a long chain with a large intracellular domain and a smaller accessory chain.

Recently, members of a series of orphan receptors with significant homology to the extracellular portions of the IL-10 and IFN receptors were assigned

functionally to individual members of the cellular IL-10 cytokine family (Fig. 3). In the case of the receptor for IL-20, the long subunit IL-20R1 (synonyms CRF2-8, ZcytoR7 and CPNM-1) is joined by IL-20R2 (DIRS-1) to form functional IL-20 receptors on the surface of keratinocytes [24]. In the case of the receptor for IL-22/IL-TIF, the long chain IL-22R (CRF2-9 or ZcytoR11) [21,39] is complemented by IL-10R2, which thus, might play a role as a common chain in different cytokine receptors, in a similar manner to the common  $\gamma$ -chain in the receptors for IL-2, IL-4 and others. Another receptor for IL-22/IL-TIF lacks a transmembrane and intracellular domain. Because this soluble factor is able to bind to IL-22/IL-TIF and neutralize its function, this molecule has been termed IL-22-binding protein (IL-22BP; synonyms CRF2-10 and IL-22RA2) [40–42]. Recently, IL-19, IL-20 and IL-24/MDA-7 have been assigned to additional CRF2 heterodimers [43]. The previously assumed IL-20-specific IL-20R1/IL-20R2 heterodimer is also used by IL-19 and IL-24/MDA-7. In addition, IL-20 and IL-24/MDA-7, but not IL-19, bind to IL-22R/IL-20R2 complexes (Fig. 3b) [43]. Thus, the CRF-2 members show some degree of promiscuity.

Signaling through the heterodimeric CRF2 complexes for the individual IL-10 paralogs involves the activation of Jak kinases and the phosphorylation of signal transducer and activator of transcription (STAT) factors, which induce  $\gamma$ -activated sequence (GAS)-dependent or otherwise STAT-dependent transcription, for example of the gene encoding suppressor of cytokine signaling 3 (SOCS-3) [41]. Alternative signaling pathways have not been investigated yet. Because STAT3 seems to be a common major transcription factor mediating stimulatory effects, the tissue-specific surface expression of the specific combination of CRF2 subunits is likely to play the key role in determining the function of the respective IL-10 family member.

#### Biological activities of IL-10-related proteins

Because IL-10 had been shown to act as a survival and differentiation factor for B cells, the highly homologous EBV IL-10 was thought to be a key factor for viral B-cell transformation [2,3]. However, cellular IL-10 was much more relevant functionally in EBV-transformed B cells, and the lytically expressed viral gene encoding IL-10 was dispensable for the transforming function [5,6]. B cells that were transformed by a deletion mutant virus were unable to suppress the secretion of IFN- $\gamma$  by autologous peripheral-blood cells [6]. Although many inhibitory functional similarities were observed between cellular and EBV IL-10, several stimulatory functions of cellular IL-10 could not be detected with the homolog from EBV (reviewed in Ref. [1]). The IL-10R1 binding affinity of EBV IL-10 was considerably lower than for cellular IL-10; however, the stimulatory effect on IL-10R1-transfected target cell lines was

similar [4]. A single amino acid exchange (Ile87Ala) abolished the stimulatory phenotype of the cellular cytokine, but not the inhibitory functions shared with EBV IL-10 [44]. The reduced receptor affinity and absence of stimulatory functions suggest that local immunosuppression by EBV IL-10 might be a relevant factor during EBV infection. Also, the highly homologous IL-10 variant from the parapoxvirus Orf, similarly to cellular ovine IL-10, was able to stimulate proliferation in a sheep thymocyte proliferation assay and inhibit cytokine secretion [8,9], indicating that the Orf IL-10 uses the classical IL-10 receptor also.

By contrast, primate cytomegaloviruses (HCMV and SCMV) of humans, and macaques, baboons and African green monkeys, respectively, encode IL-10 proteins with a low degree of sequence conservation (27%) [10,11]. The viral IL-10 open reading frame had been missed before because the encoding UL111a gene contains two introns. In spite of the low sequence homology, CMV IL-10 is able to bind to and signal through the classical IL-10R1/IL-10R2 receptor complex and compete with cellular IL-10 for binding sites [10]. Correspondingly, most of the amino acid residues that are considered crucial for receptor binding are conserved in CMV IL-10. It is tempting to speculate that CMV IL-10 might cause local, or even systemic, immunosuppression during CMV infection, thus providing an additional immune-evasion function for this virus.

Generally, the paralogous IL-10-related proteins share only limited homology with IL-10 (25% identity and 50% similarity on average) (Tables 1,2 and Fig. 1). In contrast to CMV IL-10, which shows a similarly low degree of conservation, the receptor usage and receptor-binding residues are variable for the cellular paralogs (Fig. 2). Whereas many immunosuppressive and immunostimulatory functions have been described for IL-10 (reviewed in detail in Ref. [1]), the biological activities of the cellular paralogs are strictly distinct from those of IL-10, at least as far as is known for IL-20, IL-22/IL-TIF and IL-24/MDA-7. Moreover, the site of expression is distinct for each individual IL-10 paralog. Whereas IL-10 is produced by a wide variety of cell types, including Th2 cells, macrophages and keratinocytes, the expression patterns are more restricted for the other family members (Table 3). IL-26/AK155 is transcribed by monocytes and various types of T cells [22]; IL-22/IL-TIF is produced primarily by CD4<sup>+</sup> T cells [19,20]; IL-24/MDA-7 is transcribed in differentiated melanocytes, lipopolysaccharide-stimulated monocytes, blood cells and Th2 cells after stimulation with anti-CD3 antibodies and IL-4 [13,15,45]; and IL-19 is transcribed by lipopolysaccharide-treated monocytes and various sorts of B cells [25] (Table 3). By contrast, the site of expression is still unclear for IL-20, transcripts of which have been found at low levels only in some tissue samples from skin and trachea [24].

Table 4. Overview of the cellular members of the interleukin-10 family<sup>a</sup>

	IL-10	IL-19	IL-20	IL-24/MDA-7	IL-26/AK155	IL-22/IL-TIF
<b>Expression</b>	Leukocytes and skin	B cells and monocytes	Skin and trachea	PBMCs and melanoma	T cells	T cells
<b>Induced by</b>	IL-12, LPS and others	LPS or GM-CSF	–	IFN- $\beta$ and mezerein	Transformation by herpesvirus saimiri	IL-9 or LPS
<b>Receptor</b>	IL-10R1/IL-10R2	IL-20R1/IL-20R2	IL-20R1/IL-20R2 or IL-22R/IL-20R2	IL-20R1 and IL-20R2 or IL-22R/IL-20R2	–	IL-22R/IL-10R2 or IL-22BP
<b>Signaling</b>	STAT1 and STAT3	STAT3	STAT3	STAT3	–	STAT1 and STAT3
<b>Target tissue</b>	Leukocytes	–	Skin	Tumor cells	–	Liver
<b>Biological effects</b>	Many	–	Skin differentiation and keratin expression	Tumor and apoptosis inhibition	–	–
<b>Model target cell line</b>	Molt-4 and others	–	HaCaT	HeLa, T47D and MCF-7	–	HepG2, MES-13, PC-12, TK-10 and SW-480
<b>Proposed disease association</b>	Autoimmunity	–	Psoriasis	Melanoma	–	Acute-phase liver reaction

<sup>a</sup>Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN- $\beta$ , interferon  $\beta$ ; IL-22BP, IL-22 binding protein; IL-TIF, interleukin-10-related T-cell-derived inducible factor; LPS, lipopolysaccharide; MDA-7, melanoma differentiation-associated antigen 7; PBMCs, peripheral-blood mononuclear cells; STAT, signal transducer and activator of transcription.

The IL-24/MDA-7-encoding cDNA was isolated initially as a melanoma differentiation-associated antigen from cultured human melanoma cells after differentiation treatment with IFN- $\beta$  and the protein kinase C activator mezerein, which leads to transcriptional activation by AP-1 and C/EBP and an increased half-life of the transcript [13,46,47]. Gene transfection reduced the proliferation and colony formation of melanoma and other tumor cells, but not nontransformed cells [13,14]. The inhibition of proliferation of mammary carcinoma cells by IL-24/MDA-7 was shown to be mediated by the induction of apoptosis and increased levels of expression of the proapoptotic protein BAX [17]. Moreover, the IL-24/MDA-7 ortholog from rats has been described to be induced by activated Ras and transcribed in fibroblasts during wound healing [16,18].

IL-22/IL-TIF is secreted primarily by activated CD4<sup>+</sup> T cells [19,20]. The target-cell types include mesangial, neuronal and hepatoma cells (Table 4). In hepatoma cells or after injection into the mouse liver, the production of acute-phase reactive proteins is induced, among them serum amyloid A,  $\alpha$ 1-antichymotrypsin and haptoglobin [20]. The systemic effects of IL-22/IL-TIF resemble acute-phase response symptoms, including anemia, increased numbers of platelets, decreased levels of serum albumin, increased levels of serum amyloid A and fibrinogen, and decreased body weight. In addition, basophilia of proximal renal tubules was reported [48].

IL-20 was shown to target keratinocytes that express the specific heterodimeric receptor (IL-20R1/IL-20R2). Psoriatic skin was reported to up-regulate its expression of both chains of the IL-20 receptor. When IL-20 was overexpressed in transgenic mice, neonatal lethality as well as severe skin abnormalities, including hyperkeratosis,

hyperproliferation and aberrant epidermal differentiation, were observed [24,49].

#### Therapeutic application of IL-10 homologs

Whereas cellular IL-10 has been studied in numerous experimental therapeutic approaches (summarized in Ref. [1]), only two other members of the IL-10 family, namely EBV IL-10 and IL-24/MDA-7, have been used in preclinical gene-therapy experiments.

EBV IL-10 has a high therapeutic potential for immunosuppression because it shows most of the inhibitory, but only few of the stimulatory, functions of cellular IL-10. Thus, the application of EBV IL-10-expressing gene vectors for organ transplantation therapy is a promising approach. In an initial study, a local anergy to allogeneic and syngeneic mouse tumors was shown after retroviral transduction of the gene encoding EBV IL-10 [50]. Subsequently, a similar strategy was successful for prolonging the survival of ectopic cardiac allografts in mice [51]. Alternatively, the lipid-mediated gene transfer of EBV IL-10 by perfusion of vascularized allografts led to prolonged graft survival and diminished host immune responses [52]. Adenoviral expression vectors for EBV IL-10 gave prolonged transgene expression in murine lungs after intratracheal administration and a reduced inflammatory reaction. In addition, adenoviral EBV IL-10 vectors have been used successfully in mice for reducing the symptoms of zymosan-induced multiorgan failure and necrotizing pancreatitis [53,54].

IL-24/MDA-7, as a tumor suppressor gene, offers a promising strategy for tumor therapy, according to the observations that transfected tumor cells stopped proliferating, lost tumorigenicity and underwent apoptosis, whereas normal nonmalignant cells remained unaffected [13,17,55]. In subsequent studies, adenoviral overexpression of IL-24/MDA-7 protein suppressed proliferation significantly in a variety of

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human tumor-cell lines, irrespective of the p53 status of the tumor, mediated by caspase activation [56,57]. At least after adenoviral transduction, soluble IL-24/MDA-7 protein was detectable in the tissue-culture supernatant [57], which might suggest an autocrine or paracrine receptor-mediated mechanism.

#### Concluding remarks

Although the various members of the IL-10 cytokine family show unequivocal structural

homology, the diverse biological effects of the cellular paralogs are most probably controlled by the tissue-specific expression of ligand-specific heterodimeric receptors. The list of proposed disease associations, such as tumor induction through loss of suppressor expression, acute-phase responses or inflammatory skin diseases such as psoriasis, is unlikely to be complete. The same applies to our knowledge of possible therapeutic applications.

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# Stat1-dependent and -independent pathways in IFN- $\gamma$ -dependent signaling

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The paradigm that emerged from studies during the past decade established a central role for Jak–Stat (signal transducer and activator of transcription) signaling pathways in promoting the diverse cellular responses induced by interferon  $\gamma$  (IFN- $\gamma$ ). However, recent studies have shown that the IFN- $\gamma$  receptor activates additional signaling pathways and can regulate gene expression by Stat1-independent pathways. The diversity of gene-expression patterns mediated by Stat1-dependent and -independent mechanisms and the balance between these two pathways play an important role in the biological response to IFN- $\gamma$ .

Activated T cells and natural killer (NK) cells produce interferon  $\gamma$  (IFN- $\gamma$ ), a pleiotropic cytokine involved in antiproliferative and antiviral responses, immune surveillance and tumor suppression [1,2]. IFN- $\gamma$  is the major means by which T cells activate macrophages. The complex genetic programs elicited by IFN- $\gamma$  in the immune system, as well as outside the immune system (e.g. in fibroblasts and epithelial cells), account for the diverse activities of IFN- $\gamma$  in mediating host defense and immunopathology. Biological responses to IFN- $\gamma$  are mediated mainly by the regulation of gene expression. It has been established that the majority of the pleiotropic effects of IFN- $\gamma$  is owing to several gene products that are regulated by the Jak–Stat1 (signal transducer and activator of transcription 1) pathway [3]. We summarize recent advances in the understanding of IFN- $\gamma$ -mediated signal transduction and the use of microarrays for the study of IFN- $\gamma$ -dependent regulation of gene expression. The results reveal that both Stat1-dependent and -independent pathways

play important roles in the full range of biological responses to IFN- $\gamma$ .

The Jak–Stat1 pathway in IFN- $\gamma$ -dependent signaling IFN- $\gamma$  exerts its effects on cells by interacting with a specific receptor composed of two subunits, IFN $\gamma$ R1 and IFN $\gamma$ R2, that is expressed on nearly all cell surfaces. Binding of IFN- $\gamma$  to its receptor induces receptor oligomerization and activation of the receptor-associated Janus kinases Jak1 and Jak2 by trans-phosphorylation. The activated Jaks phosphorylate the intracellular domain of the receptor (e.g. tyrosine 440 of human IFN $\gamma$ R1), which serves as a docking site for Stat1 [2]. Additional tyrosine residues of the receptor and the Jaks themselves are phosphorylated also. Stat1 is phosphorylated on tyrosine 701, undergoes dimerization through Src-homology-2 (SH2) domains, translocates to the nucleus and regulates gene expression by binding to  $\gamma$ -activated sequence (GAS) elements in the promoters of IFN- $\gamma$ -regulated genes [4,5]. At some point during the early phase of activation, Stat1 is phosphorylated also on serine 727 by a process involving phosphatidylinositol 3-kinase and Akt [6] that is required for maximal transcriptional activity [7]. The physiological relevance of IFN-dependent signaling through the Jak–Stat pathway has been established by generating and characterizing mice with a targeted disruption of the gene encoding Stat1 [8,9]. Stat1-null mice develop normally, but lack many classical

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