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Viral and cellular interleukin-10 (IL-10)-related cytokines: from structures to functions

Abstract

Full text

INTRODUCTION

Interleukin-10 (IL-10) is a major anti-inflammatory cytokine, originally described as a factor inhibiting IFN-gamma production by murine Th1 lymphocytes [1]. Because IL-10 was preferentially produced by T cells from the Th2 subtype, and was found to be a potent inhibitor of IFN-gamma production by Th1 cells, it was

proposed to act as a Th2 promoting cytokine. More recently, IL-10 was found to be produced by another subset of T lymphocytes called regulatory T cells (Treg) and which are supposed to play a key role in inhibiting autoimmune processes [2]. All these observations point to IL-10 as a major player in the regulation of the T lymphocyte network.

Later on, the ability of IL-10 to inhibit cytokine production by T cells turned out to be indirect, *via* inhibition of accessory cells [3]. IL-10 acts as an inhibitor of activated macrophage/monocyte functions, such as cytokine synthesis, NO production, MHC class II expression and costimulatory molecule expression [4-8]. Monocytes and macrophages do not only represent a major target of IL-10, they are also responsible for a large part of IL-10 production, particularly during inflammatory processes [5, 9]. The importance of IL-10 in inflammation has been demonstrated in various experimental settings. In experimental endotoxemia models, IL-10 inhibited TNF production and rescued Balb/c mice from LPS-induced toxic shock [10, 11]. Conversely, mice treated with anti-IL-10 antibodies are highly susceptible to low doses of LPS [12]. IL-10-deficient mice also illustrate its role in inflammation as they are characterized by a spontaneous enterocolitis, triggered by resident enteric bacteria [13].

VIRAL IL-10 HOMOLOGS

The sequencing of the human IL-10 cDNA led to the observation that the genome of the Epstein-Barr virus (EBV) contained a very similar open reading frame called BCRF1 [14, 15], showing 84% amino acid identity for the mature protein. BCRF1 has subsequently been renamed viral IL-10 (vIL-10), and was shown to be expressed during the lytic phase of virus infection [16]. Interestingly, vIL-10 exhibits only a subset of IL-10 activities, although it is supposed to bind to the same receptor complexes (but with a significantly lower affinity than human IL-10) [17-19]. It is tempting to speculate that vIL-10 is used by EBV to escape the immune system, or to favor B cell proliferation and transformation. However, experimental evidences for a role of vIL-10 in these processes are still lacking.

IL-10 gene homologs have also been described in other viruses such as equine Herpes virus type 2 (EHV2) [20], and in the Orf poxvirus [21]. The viral genes have no intron, and show a high level of sequence identity with endogeneous IL-10 from their respective hosts, horse and sheep. By contrast, a cytomegalovirus

(CMV) IL-10 gene has been described with only 27% amino acid identity [22, 23]. However, this CMV IL-10 has conserved the ability to bind the IL-10 receptor. Another characteristic of the CMV IL-10 gene is the presence of introns at positions of introns 1 and 3 of the human IL-10 gene, while the other viral IL-10 genes lack intervening sequences [22]. More recently, the sequencing of the Yaba-like disease poxvirus (YLDV) revealed the existence of another IL-10 homolog with only 30% amino acid identity [24]. The biological properties and receptor binding activity of this protein have not been described yet.

CELLULAR IL-10 HOMOLOGS

Five human IL-10-related genes, showing 20 to 35% amino acid identity with IL-10 or between each other, have been described so far. Three of them, melanoma differentiation antigen-7 (mda-7), IL-10-related T cell derived inducible factor (IL-TIF/IL-22), and AK155, were identified by cDNA subtraction cloning in various experimental models [25-27]. Two additional genes, IL-19 and IL-20, were identified when the complete human genome sequence became available [28, 29]. A philogenetic tree of the IL-10 homolog family is presented in Figure 1, together with IFN genes, which are also related to this family.

mda-7 is expressed by melanoma cell lines when differentiation is induced by IFN-beta and mezerein, a PKC activator. Although the sequence homology between mda-7 and IL-10 (24% amino acid identity) was rapidly observed, the mda-7 protein was initially not considered to be a secreted protein with cytokine activities [30]. The rat ortholog of mda-7 was called c49a [31], when it was cloned as a gene transiently upregulated during wound repair. The very same gene was designated mob-5, when it was cloned as a gene expressed by fibroblasts upon ras-transformation [32]. Finally the mouse mda-7 ortholog has been described as a gene induced by IL-4 during Th2 lymphocyte differentiation, and proposed to be called IL-4-induced secreted protein (FISP) [33]. According to the Human Genome Nomenclature committee, this gene should eventually be renamed IL-24.

IL-TIF was originally identified as a gene induced by IL-9 in murine T lymphocytes and showing 22% amino acid identity with IL-10 [26]. The IL-TIF gene is located on mouse chromosome 10 in the vicinity of IFN-gamma. This gene is duplicated in some mouse strains such as 129 or FVB/N [34]. The two copies from 129 mice, called IL-TIFalpha and IL-TIFbeta, show 98% nucleotide identity in the coding

region, and the putative proteins differ only by 3 amino acids. However, the IL-TIFbeta gene also differed from IL-TIFalpha by a 658 nucleotide deletion, including the TATA box of the promoter and the first non-coding exon. This deletion of the promoter suggests that the IL-TIFbeta gene is a pseudogene [34]. In line with this hypothesis, we failed to detect any IL-TIFbeta transcript so far, and IL-TIFalpha deficient mice do not seem to express any IL-TIF mRNA (L. Dumoutier, unpublished data). Human IL-TIF shows 79% amino acid identity with its murine ortholog, and 23% amino acid identity with human IL-10 [35]. The IL-TIF gene is located on chromosome 12q15 at 90Kb from the IFN-gamma gene and at 27Kb from AK155, which codes for another IL-10 related cytokine. The IL-TIF gene has been successively proposed by others to be recalled IL-21 and IL-22 [36]. For clarity, we will use the IL-TIF/IL-22 designation in this review.

The AK155 protein consists of 171 amino acids with 24% identity with IL-10. AK155 is expressed in T cells upon transformation by Herpes virus saimiri [27]. No murine ortholog was described so far, even by extensive screening of mouse genomic databases. This raises the possibility that this gene has not been evolutionary conserved, at least in the mouse.

Additional IL-10 homologs, namely IL-19 and IL-20 were discovered by screening EST databases, and sequencing of the IL-10 locus on chromosome 1q32, which also includes the mda-7 gene. The IL-19 protein consists of 177 amino acids showing 28% identity with IL-10, and 75% identity with its mouse ortholog [28]. The IL-20 protein consists of 176 amino acids, presenting 28% identity with IL-10, and 76% with its mouse ortholog [29]. The IL-10, IL-20, mda-7 and IL-19 genes were located within 150 kb on chromosome 1q32. Their mouse orthologs are also located in the same cluster on mouse chromosome 1.

STRUCTURAL CHARACTERIZATION OF IL-10 HOMOLOGS

X-ray crystallographic analyses of hIL-10 and vIL-10 demonstrated that IL-10 is a noncovalent symmetric homodimer that is topologically similar to the structure of IFN-gamma, both molecules forming V-shaped dimers [37, 38]. Each monomer is characterized by two disulfide bonds and by six alpha helices. Four helices (A-D) originate from one subunit, whereas the C terminal two helices (E-F) interpenetrate into the N-terminal four helix subunit (A'-D') of the dimerization partner. An engineered hIL-10 was generated by inserting six amino acids in the DE loop of the human protein, between residues 116 and 117, in order to favor

monomer formation [39]. Such a modified IL-10 molecule behaves as a monomer in gel filtration chromatography, and retains biological activity, although with reduced affinity and lower specific activity in a biological assay. A monomeric form of IL-10 could also be generated by reduction of the C62-C114 disulfide bond [40].

The crystal structure of IL-10 bound to the extracellular domain of IL-10Ralpha was recently described [41]. In solution, the IL-10/sIL-10Ralpha complex is composed by two IL-10 dimers and four sIL-10R molecules. A total of 27 residues from IL-10 make contact with IL-10Ralpha. Such residues are found mainly in helix A, the A-B loop, and helix F of IL-10. Among these residues, only nine are conserved in the CMV IL-10 homolog, which binds to the same receptor as cellular IL-10.

The amphipatic pattern of hydrophobic and hydrophilic residues for the six helices is relatively well conserved in all IL-10 homologs. IL-TIF/IL-22 was recently crystallized and is indeed composed of six alpha-helices (Nagem, R.A. *et al.* submitted for publication). However, unlike IL-10, IL-TIF/IL-22 is produced as a monomer. The basic reason for this difference stems from the composition of the loop between the D and E helices. There are 2 cysteine residues, Cys-108 and Cys-114, at the IL-10 D-E loop, which form 2 disulfide bonds, with residues Cys-12 and Cys-62, respectively. These two S-S bridges restrict the flexibility of the amino acid chain and the length of the D-E loop in such a manner that helices E and F can not fold into their respective monomer, to occupy the position of their counterparts E' and F'. This leads to intertwined dimer formation. In IL-TIF/IL-22, only one disulfide bond exists (Cys-40-Cys132) at the D-E loop. This allows for sufficient flexibility and extension of the loop to bring helices E and F in a close interaction with the helices A to D, and to complete the folding of the monomer. A second disulfide bond, in the C-terminus of helix F (Cys89-Cys178), stabilizes the monomeric IL-22 structure. The respective helices and disulfide bridges found in IL-10 and IL-TIF/IL-22 structures are schematically illustrated in Figure 2.

The tridimentional structure of the other IL-10 homologs has not been determined yet. The AK155 protein has conserved the 4 cysteine residues present in IL-10, and might therefore be produced as a dimer as well. By contrast, the mda-7 protein does not include any cysteine residue homologous to the last 2 cysteines of IL-10 (Figure 2). Thus, although it contains 2 cysteines, mda-7 can

not form any of the 2 disulfide bridges found in IL-10. The consequences of this particularity for the mda-7 structure remain unknown. IL-19 and IL-20 have the highest degree of homology amongst IL-10-related cytokines, with 37% amino acid identity. Both proteins show in addition to the 4 cysteines conserved in IL-10 and AK155, 2 additional cysteine residues in helix C and in the D-E loop. Based on the IL-10 structure, if the 2 additional cysteins are disulfide bound, it has been suggested that IL-19 and IL-20 would be monomeric, a prediction reportedly supported by analysis of recombinant IL-20 [29], but for which experimental data are still lacking.

Beside cysteine residues, 2 conserved motifs are located in helix C and in helix F respectively. The helix F conserved motif contains three residues from the IL-10Ralpha-binding epitope of IL-10 (K-138, E-142 and D-144), an epitope which also includes the conserved D-41 residue. Interestingly, three of these amino acids are also present in the IFN-gamma protein. Both for IFN-gamma and IL-10, they form a binding pocket for a tyrosine from their respective receptor, suggesting that all these ligands use similar receptor binding mechanisms [41]. By contrast, the first 20 amino acids of the mature protein are extremely different, not only between the various IL-10 paralogs, but also between human and mouse IL-TIF/IL-22. Based on the crystal structures, this N-terminal region forms a tail preceeding the first cystein and helix A, and which does not play any role in the structure or in the activity of the respective cytokines.

A sequence of 12 amino acids in the putative helix D from IL-19 and IL-20 is perfectly conserved in these proteins, but not in other IL-10 homologs. Together with the conserved cysteine pattern, this motif suggests that IL-19 and IL-20 share a very similar structure. This hypothesis has been recently confirmed with the characterization of their receptor complexes, as described below.

RECEPTOR USAGE AND SIGNAL TRANSDUCTION

The active IL-10 receptor signaling complex is composed of two different receptor chains, IL-10Ralpha and IL-10Rbeta (also called CRF2-4) belonging to the class II cytokine receptor family [42, 43]. The extracellular domains of class II cytokine receptors have tandem fibronectin type III domains, and share approximately 20% amino acid identity. By contrast, their cytoplasmic domains vary in length, and do not demonstrate any similarity in their primary structure. The subunits of the type I IFN receptor (IFNAR1 and IFNAR2), and of the type II IFN

receptor (IFNGR1 and IFNGR2), as well as Tissue Factor, a protein involved in coagulation, are also members of this family [44].

When the CRF2-4/IL-10Rbeta protein was demonstrated to be involved in IL-10 signaling, it was rapidly noticed that its expression pattern extends much beyond IL-10-target cells. This suggested that this protein could be involved in other cytokine receptor complexes. Indeed, it was later shown that the IL-10Rbeta chain is also used by IL-TIF/IL-22. First, an antibody against IL-10Rbeta blocked the biological activities of IL-TIF/IL-22 [35]. Secondly, transfection with the IL-10Rbeta cDNA conferred the ability to bind radiolabelled IL-TIF/IL-22 [45]. Thirdly, an IL-22-dependent STAT activation was observed in cells transfected with the cDNAs for both IL-10Rbeta and an orphan class II cytokine receptor, previously called ZcytoR11/CRF2-9, and proposed to be renamed IL-22R [36, 45]. Although IL-10 and IL-TIF/IL-22 share a common receptor chain, to which IL-TIF/IL-22 binds directly, these cytokines do not exhibit any antagonist activity against each other.

IL-20 binds to receptor complexes of 2 types. In an initial report, a first type of IL-20R complexes was described by associating 2 orphan receptors: ZcytoR7/CRF2-8, further renamed IL-20Ralpha, and DIRS-1, further renamed IL-20Rbeta [29]. More recently, a second type of IL-20 receptor complex was described by associating IL-20Rbeta with IL-22R [46]. The respective role of these different receptor complexes in IL-20 activities remains to be established. Interestingly, these receptor complexes are not restricted to IL-20. mda-7 has been shown to bind both types of IL-20 receptors, while IL-19 only binds to IL-20 receptors of type 1, consisting of IL-20Ralpha and IL-20Rbeta [46]. The AK155 receptor complexes have not been characterized so far.

Like the genes encoding the ligands, most of the genes encoding the class II cytokine receptors tend to be clustered in the genome (Table 1). The genes encoding IL-22R, an orphan receptor of this family and Tissue Factor are located on chromosome 1, IL-20Ralpha and IFNGR1 on chromosome 6q24, IFNAR1, IFNAR2, IFNGR2 and IL-10Rbeta on chromosome 21q22. As for the IL-10R and IL20Rbeta genes, they are located on chromosome 11q23 and 3q22, respectively.

Further analysis of the genome sequence at the 6q24 locus, showed the presence of another member of this family at 35 kb from the IFNGR1 gene. Surprisingly, when the corresponding cDNA was cloned, the predicted protein

was found to contain a classical extracellular domain from a cytokine receptor, but no potential cytoplasmic nor transmembrane domain. This soluble receptor has been called IL-22BP because it binds IL-TIF/IL-22, and neutralizes its activity [47-49]. Alternative names for the same protein include ZcytoR16, CRF2-10 and IL-22RA2. The different receptor complexes composed of class II cytokine receptors are schematically represented in Figure 3.

Most of signal transduction studies dealing with class II cytokine receptors have focused on IFN receptors, which will not be discussed here as excellent reviews are available on this topic [50, 51]. By contrast, signaling through the receptors for the IL-10 homologs has been poorly investigated so far, with the exception of the IL-10 receptor itself. Stimulation with IL-10 leads to the Tyr-phosphorylation of the Tyrosine kinases Jak1 and Tyk2, which are constitutively associated with IL-10Ralpha and IL-10Rbeta, respectively [43, 52]. The cytoplasmic domain of IL-10Rbeta is relatively short and its role in signaling might be restricted to the recruitment of Tyk2. The IL-10Ralpha chain has a larger cytoplasmic domain and becomes phosphorylated on at least 2 tyrosine residues, which are used as docking sites for recruiting STAT3 [53, 54]. STAT3 is then phosphorylated, dimerizes, and migrates to the nucleus to trigger gene expression. In addition to STAT3, STAT1 and STAT5 have also been reported to be activated in response to IL-10, but their role in IL-10 activities remains unclear [55, 56]. So far, IL-10 signaling does not seem to be affected by dominant negative STAT1 or STAT5, nor by mutating the STAT1 gene [57, 58].

By contrast, STAT3-deficient macrophages are completely refractory to the effects of IL-10, namely to inhibition of proliferation and to inhibition of cytokine production [59, 60]. However, a dominant negative form of STAT3, which lacks the transcriptional activation domain, blocks only the anti-proliferative activity, but not the inhibition of cytokine production [57]. Altogether, these observations suggest that STAT3 might play distinct roles in IL-10 signaling. Inhibition of proliferation might be mediated by transcriptional upregulation of cell cycle inhibitors p21-KIP1 and p19-INK4D [61], whereas non-transcriptional mechanisms could be involved in the inhibition of cytokine production [60]. The latter activity also requires 4 serine residues out of the 15 C-terminal amino acids of IL-10Ralpha, a region which is dispensable for STAT3 activation, suggesting that another pathway might cooperate with STAT3 in this process [59].

In addition to the STAT pathway, IL-10 was also shown to activate the PI3-kinase and p70 S6 kinase. Although inhibitors of this pathway could block IL-10-induced proliferation of a murine mast cell line, they failed to affect the effect of IL-10 on cytokine production by activated macrophages [62]. Other signaling pathways such as MAP kinases or NF-kappaB can be inhibited by IL-10 [63-66], but the mechanisms underlying this effect remain to be unraveled.

Little is known about signal transduction in response to the other IL-10 homologs. A common feature for IL-19, IL-20, IL-22 and mda-7 is the activation of STAT3 [46], but other pathways could be activated as well. In this respect, preliminary results indicate that IL-TIF/IL-22 activates the Erk and p38 MAP kinase pathways in hepatocytes (Lejeune *et al.* manuscript in preparation).

Tissue Factor differs from the other members of this family both for its binding specificity and for its signaling pathway [67, 68]. This molecule is the principal initiator of the extrinsic coagulation pathway, *via* the binding of Factor VII followed by activation of Factor X and further haemostasis. It has been shown that Tissue Factor-Factor VII interaction induces the activation of c-src, lyn and yes tyrosine kinases and subsequently of PI3-kinase, PKB, rac GTPases, as well as Erk and p38 MAP kinases [68]. JAK kinases have not been yet demonstrated to be involved in Tissue Factor mediated activities, and it is not known whether the signaling cascade triggered by this receptor-like protein is related or not to classical cytokine receptors.

CELLULAR SOURCES AND BIOLOGICAL ACTIVITIES

Although it binds to the same receptor, the EBV-derived viral IL-10 does not share all the activities of cellular IL-10. EBV vIL-10, like human IL-10 inhibits macrophage activation and promotes B cell survival and proliferation [15, 18, 69, 70]. By contrast, EBV vIL-10 does not compare with its cellular homolog for enhancing class II MHC expression on mouse B cells, or for promoting mouse thymocyte and mast cell proliferation [18, 69, 71]. Its ability to inhibit IL-2 production by activated $CD4^+$ T cells is also greatly reduced, as compared to human IL-10 [19]. However, the latter activity was restored by increasing vIL-10 concentration, in agreement with its lower affinity for the human IL-10 receptor. The molecular basis for this selective loss of biological activities remains poorly understood. On the one hand, the threshold of receptor activation might vary from one cell type to another, and the lower affinity of vIL-10 for the IL-10 receptor could

explain the discrepancy for some activities. On the other hand, differences in the ligand/receptor interaction might also result in qualitative differences in the downstream signaling cascade.

Interestingly, a change of a single amino acid at position 87, isoleucine in human IL-10 or alanine in EBV IL-10, was reported to be responsible for most of the difference in activities [72]. In this study, variations in specific activities did not perfectly correlate with changes in affinities for the receptor, depending on the cell lines used as bioassays, suggesting that affinity differences fall short to explaining the discrepancies between human and EBV IL-10. Independently from the structural basis responsible for this situation, it is tempting to speculate that the EBV IL-10 represents a selectively mutated IL-10 variant that benefits viral pathogenesis by exerting the immunosuppresive activities of IL-10, without its immunostimulatory properties.

The IL-10-related gene of the parapoxvirus Orf is able to stimulate proliferation of sheep thymocytes and to inhibit cytokine secretion similarly to cellular ovine IL-10 [21, 73], and unlike its EBV counterpart has conserved the isoleucine corresponding to position 87 of human IL-10. Although the CMV IL-10 shows only 27% amino acid identity with human IL-10, this molecule binds to the human IL-10 receptor complex, when reconstituted in transfected hamster cells [22]. However, the affinity for the receptor, as well as the activity of CMV IL-10 in the various IL-10 bioassays have not been reported yet.

In contrast to the viral IL-10 homologs, which use the *bona fide* IL-10 receptor complexes, the cellular IL-10-related cytokines use distinct signaling complexes, as already mentioned. Thus, despite their structural relationship, it must be stressed that none of the IL-10 paralogs recapitulates the anti-inflammatory activities of IL-10. However, all these genes appear to be upregulated by inflammatory stimuli, and each of them is likely to play a role at some stage of the inflammatory process.

IL-TIF/IL-22 was discovered by searching for IL-9 inducible genes in mouse T lymphoma cells [26]. The IL-TIF/IL-22 induction was maximal after 2 hours, did not require protein synthesis, and involved an increase of the IL-TIF promoter activity mediated by STAT transcription factors [34]. IL-TIF/IL-22 is also induced by polyclonal activation of freshly isolated T cells using lectins or anti-CD3 antibody, and is preferentially produced by mouse Th1 cells [74], thereby sharply

contrasting with IL-10. *In vivo*, the IL-TIF/IL-22 mRNA is upregulated in most organs two hours after LPS injection, supporting a role in inflammation [35].

Various target cell types were described by screening cell lines for STAT activation in the presence of IL-TIF/IL-22. These include mesangial cells, neuronal precursors, lung and intestinal epithelial cells, melanomas and hepatoma cells. Contrasting with IL-10, IL-TIF/IL-22 fails to activate STAT transcription factors in lymphoid or myeloid cell lines *in vitro*, and does not inhibit the production of proinflammatory cytokines by monocytes in response to LPS [36]. However, modest inhibitory effects on IL-4 production from Th2 T cells were reported [36]. Further investigations are definitely needed to better determine the role of this new Th1 cytokine on T lymphocyte activation and differentiation.

In hepatoma cells, IL-TIF/IL-22 upregulates the production of acute-phase reactants, such as serum amyloid A (SAA), alpha-1 anti-chymotrypsin and haptoglobin. The same activity was observed, *in vivo* in Balb/c mice, where the SAA mRNA is increased upon injection of IL-TIF/IL-22 [35]. The effect of prolonged IL-TIF/IL-22 expression *in vivo* has been investigated by injecting an adenovirus encoding IL-TIF/IL-22 intravenously in C57BL/6 mice [74]. Such injections result in dramatic systemic effects within 2 weeks, including decreased red blood cell counts, increased platelet counts, decreased serum albumin, increased SAA and fibrinogen, and decrease in body weight. The mechanisms underlying the toxicity of prolonged IL-TIF/IL-22 expression are not yet understood, but this might result from renal lesions, as proximal renal tubules show extensive signs of basophilia, a sign of tubular cell suffering [74].

A unique feature of IL-TIF/IL-22 among the IL-10-related cytokines is the existence of a natural antagonist, called IL-22BP. This molecule was characterized independently by 3 groups based on its ability to suppress STAT activation in IL-TIF/IL-22 responsive cells [47], to suppress MHC class I antigen expression in cell transfected with a chimeric receptor between IL-22R and IFNGR1 [48], and to block the proliferation of BaF/3 cells transfected with the IL-22R [49]. This gene is constitutively expressed in various organs with the highest levels in placenta and breast [75]. Based on the toxicity of *in vivo* IL-TIF/IL-22 expression, it is tempting to speculate that the IL-22BP protein is a regulatory molecule that protects against potential damage caused by IL-TIF/IL-22 prolonged exposure.

mda-7 was initially isolated from human melanoma cells after treatment with IFN-beta and mezerein, which induce suppression of DNA synthesis and cell cycle progression, together with terminal cell differentiation [25, 76]. IFN-beta and mezerein treatment of the melanoma cells led to an increase of the half-life of the mda-7 transcript, and to a transcriptional activation involving AP-1 and C/EBP [77, 78]. In addition, the mda-7 gene seems to be expressed preferentially by normal melanocytes or by early stages of melanomas, rather than by metastatic melanomas, suggesting a role in tumor progression [25, 79].

Ectopic expression of mda-7 in melanoma cells reduced growth and inhibited colony formation. These activities were extended to other tumor cells, such as prostate cancer cells and breast tumors, but not to non-transformed cells [30]. In breast tumors, inhibition of cell proliferation was associated with induction of the proapoptotic protein BAX, followed by apoptosis [80]. Although mda-7 adenoviruses failed to induce growth suppression in pancreatic cell lines, combination of mda-7 adenoviruses with K-ras antisense oligonucleotides could induce growth suppression and apoptosis, suggesting that activated ras can protect against the proapoptotic effect of mda-7 [81].

Noticeably, all the studies designed to evaluate the potential anti-tumor activity of mda-7 involved transfection- or adenovirus-mediated gene expression, but not administration or incubation with recombinant protein, probably because mda-7 was not initially considered as a secreted factor [25, 82]. Additional studies using recombinant secreted protein are definitely needed to further analyze the mechanisms underlying this activity. In fact, the possibility remains that the mda-7 protein exists both as a secreted form, and as a cell associated form, each exerting their activity through different mechanisms. However, a recent report suggests that supernatants from mda-7 adenovirus infected cells recapitulate the growth inhibitory activity of the viral construct [83].

mda-7 expression is clearly not restricted to melanocytes and melanomas. The mda-7 rat ortholog, called mob5 or c49a, have been described to be induced by activated Ras and during wound healing [31, 32]. The mouse mda-7 ortholog, FISP, was found to be expressed by Th2 cells stimulated by IL-4, suggesting a potential role during immune responses [33].

As mentioned already, mda-7 binds to the same 2 types of receptors as IL-20 [46]. It is therefore likely that mda-7 and IL-20 exhibit very similar biological

activities. The activities of IL-20 have been analyzed *in vivo* in IL-20 transgenic mice, using various tissue-specific promoters [29]. These mice exhibit neonatal lethality with skin abnormalities including aberrant epidermal differentiation. This dramatic phenotype probably results from the activity of IL-20 on keratinocytes, which respond *in vitro* to IL-20 by STAT activation [29]. IL-19, which also binds to type 1 IL-20 receptor complexes, might share this activity with IL-20 and mda-7, but functional data are still lacking for this cytokine, which is only known to be produced by LPS- or GM-CSF-activated monocytes [28].

Finally, AK155 was discovered by searching for genes induced in Herpes saimiri-transformed T lymphocytes [27, 84]. AK155 is secreted by such transformed T cells, and by activated T cell lines or peripheral blood cells, but not by B cells. So far, the activity of this cytokine is still completely unknown.

The cellular sources and biological activities described so far for the non-viral IL-10-related cytokines are summarized in Table 2.

THERAPEUTIC APPLICATIONS

The characterization of IL-10 as an anti-inflammatory and immunosuppressive cytokine has raised major hopes for chronic inflammatory diseases such as Crohn disease, rheumatoid arthritis (RA), psoriasis and chronic hepatitis. Phase I and II clinical trials have shown that IL-10 is well tolerated without serious side effects at doses up to 25 mug/kg. IL-10 administered IV at such doses inhibited LPS-induced rises in temperature and release of cytokines in healthy human volunteers, when given 2 min before endotoxin [85]. Administration of IL-10 showed some clinical benefit in patients with chronic active Crohn disease [86]. Similarly, some efficacy was observed when IL-10 was administrated for 28 days to RA patients [87]. In psoriatic patients, IL-10 treatment significantly decreased psoriatic area and severity index [88]. Recent studies using IL-10 to suppress pathology associated with chronic hepatitis showed that IL-10 normalized serum levels of alanine aminotransferase, a marker for hepatic inflammation, and reduced liver fibrosis in over 50% of treated patients [89, 90]. By contrast, IL-10 did not reduce virus RNA level, indicating that it did not affect viral load, but instead limited pathogen-induced pathology.

These early clinical trials are encouraging, although IL-10, like many cytokines, might turn out to be a more efficient therapeutic in animal models than in human

diseases. EBV IL-10, could have a higher anti-inflammatory activity, because it seems to be devoid of the immunostimulatory effects of human IL-10. In animal models, viral IL-10 expression is consistently beneficial for cardiac allograft survival [91], pointing to EBV IL-10-expressing vectors as a promising approach for organ transplantation. Adenoviral expression vectors for EBV IL-10 showed prolonged transgene expression in murine lungs after intratracheal administration, and reduced inflammatory reactions [92, 93]. In addition, adenoviral EBV IL-10 vectors have been successfully used in mice in order to reduce the symptoms of zymosan-induced multiorgan failure and necrotizing pancreatitis [92, 93].

On the other hand, increased IL-10 production has been observed in systemic lupus erythematosus (SLE), a complex autoimmune disorder characterized by high levels of serum anti-DNA antibodies and glomerular immune complex deposition [94]. Interestingly, the activity of the disease correlates with serum levels of IL-10 [95]. When SCID mice were reconstituted with PBLs from SLE patients, anti-IL-10 antibodies could block autoantibody production [96], pointing to a major role of IL-10 in the pathogenesis of SLE. Finally, administration of an anti-IL-10 antibody to SLE patients has been reported to lead to a long lasting reduction of most disease parameters in 5/6 patients [97].

The other IL-10 homologs are still far from applications in human diseases. Based on the observation that mda-7 adenoviruses induce apoptosis in tumor cells but not in normal cells, this strategy has been proposed for tumor therapy [82]. Analysis of IL-20 transgenic mice pointed to a role for this molecule in psoriasis [29]. Electron microscopic analysis of skin from these mice showed intramitochondrial lipoid inclusions, mottled keratohyaline granules, and relatively few tonofilaments, similar to what is observed in human psoriatic skin. Expression of IL-20Ralpha and IL-20Rbeta mRNA is observed in skin, and might be upregulated in patients with psoriatic disease [29]. These observations suggest that IL-20, but also IL-19 and mda-7 which bind to this receptor, could play a role in the development of this disease. Antagonists of IL-20, IL-19 or mda-7, could be useful tools in psoriasis, if an increased activity of these cytokines can be demonstrated in the human disease.

CONCLUSION

The cytokine world has expanded dramatically during the last few years. First generation cytokines such as IL-10 were discovered based on biological activities *in vitro*, which provided us with direct clues about their physio-pathological functions. A second wave of new factors, such as mda-7, IL-TIF/IL-22 and AK155 were discovered with improvement of molecular biology techniques, allowing for the identification of genes induced under particular circumstances. Finally, the complete sequencing of the genome and its annotation provided us with a last wave of cytokine genes such as IL-19 and IL-20. Within a few years, the number of players that we have to deal with has been increased several folds. The next challenge will consist in defining the respective roles of all these orphan factors in the cytokine network, and ultimately in which diseases these molecules can be beneficial or detrimental. The hunt for discovering new cytokines is now almost over, but the hunt for determining their function is only beginning, and our present understanding of the role of IL-10-related cytokines is soon to be out of date.

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Figures

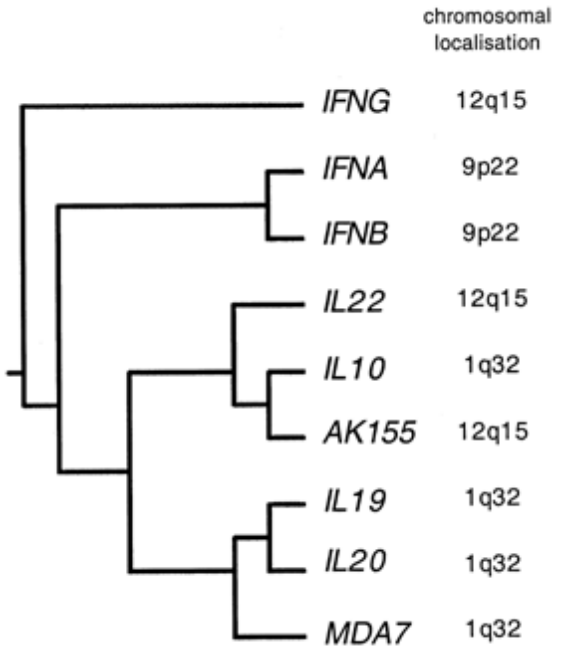


Figure 1 Phylogenetic tree of IL-10-related cytokines. Multiple sequence alignment was realized with the ClustalW software. The chromosomal localization of each human gene is indicated.

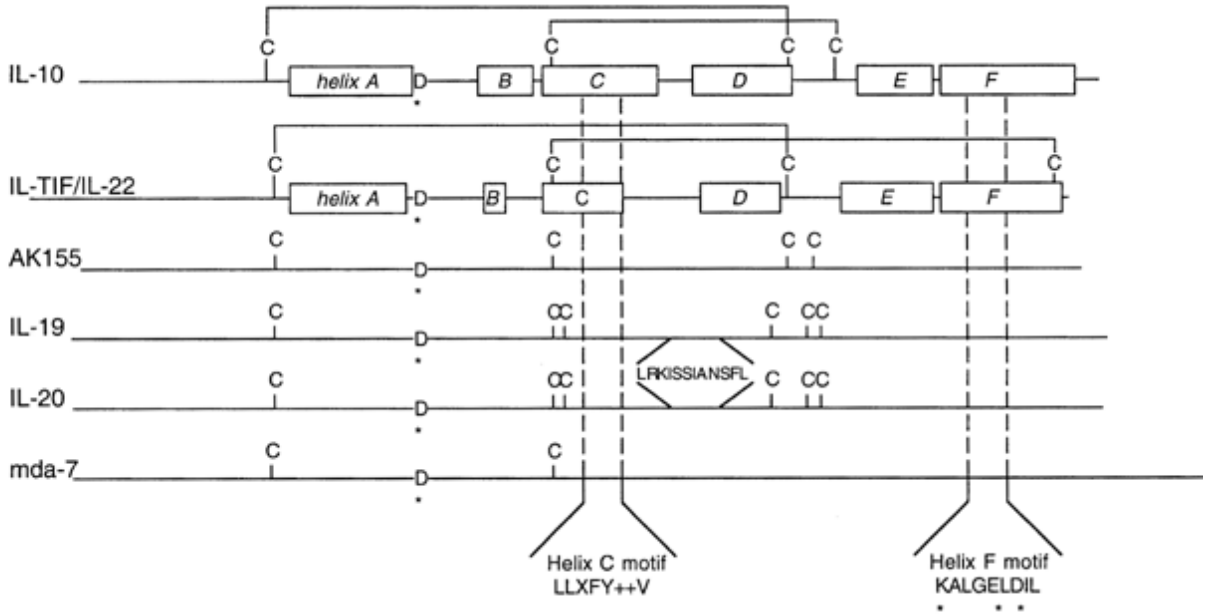


Figure 2 Schematic representation of IL-10-related cytokines. The proteins are represented by a line, on which the position of the six helices determined experimentally for IL-10 and IL-22 are shown by boxes. The residues that are conserved in the whole family are indicated. Cysteines from the mature proteins are indicated, and the disulfide bonds formed in IL-10 and IL-22 are indicated by lines. The two motifs conserved in all IL-10 homologs are shown, with positively charged residues indicated by "+". Asterisks indicate the residues that are conserved in the family among those that form the receptor binding interface in the IL-10/IL-10R1 complex. The motif conserved between IL-19 and IL-20 is also shown.

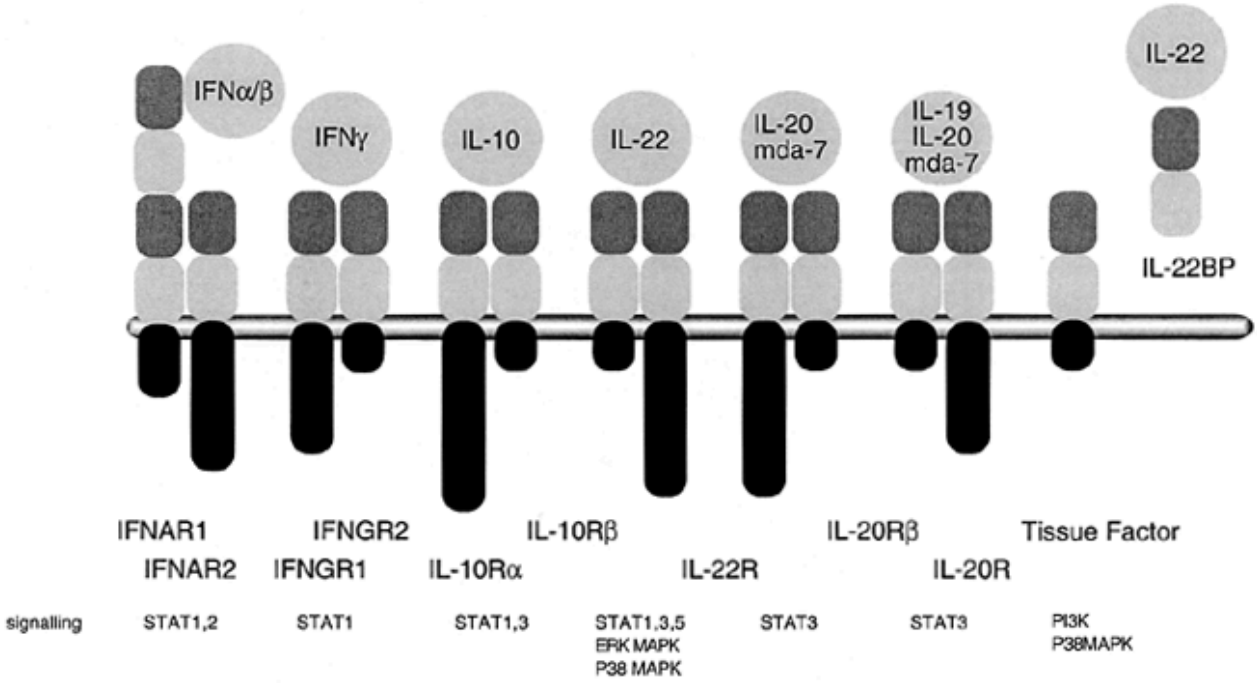


Figure 3 Schematic representation of class II cytokine receptor family and their signaling pathways.

Table 1. Genomic localization of class II cytokine receptor genes

Chromosomal localization	Gene	Alternative names
Chromosome 1p35	IL-22R	CRF2-9, ZcytoR11
	(Orphan)	LICR-2, CRF2-12
1p22	TF	CD142
Chromosome 3q22	IL-20Rb	DIRS1
Chromosome 6q24	IL-20Ra	CRF2-8, ZcytoR7
	IFNGR1	
	IL-22BP	CRF2-10, ZcytoR16, IL-22Ra2
Chromosome 11q23	IL-10Ra	
Chromosome 21q22	IFNAR2	
	IL-10Rb	CRF2-4
	IFNAR1	
	IFNGR2	

Table 2 Cellular sources, receptor usage and biological effects of IL-10-related cytokines

	Cellular source	Receptor	Biological effects
IL-TIF/IL-22	T cells stimulated by IL-9, or by > CD3 Mast cell stimulated by IL-9 All organs after LPS injection	IL-22R/IL-10Rβ	Induction of acute phase proteins in hepatocytes Renal tubular basophilia induced by adenoviral expression *in vivo*
mda-7 (human)	Melanoma cells Skin fibroblasts LPS-activated PBMCs	Type I: IL-20R/IL-20Rβ Type II: IL-22R/IL-20Rβ	Irreversible growth arrest of tumors by apoptosis and differentiation Inhibition of angiogenesis Antitumor effect *in vivo*
mob5/C49a (rat)	Epithelial cells transformed by Ha-ras or Ki-ras Wound healing		
FISP (mouse)	Th2 cells stimulated by IL-4		
IL-19	LPS- or GM-CSF activated monocytes	IL-20R/IL-20Rβ	
IL-20	LPS- or > CD3-activated PBMCs	Type I: IL-20R/IL-20Rβ Type II: IL-22R/IL-20Rβ	STAT activation in keratinocytes Neonatal lethality with skin abnormalities in transgenic mice
AK155	T cell lines, T cells infected by herpes virus saimiri Activated monocytes		

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In this issue

Viral and cellular interleukin-10 (IL-10)-related cytokines: from structures to functions

Laure Dumoutier, Jean-Christophe Renauld
IL-10, IL-19, IL-20, IL-22, mda-7, AK155.

Dendritic cells (DC) promote natural killer (NK) cell functions: dynamics of the human DC/NK cell cross talk

Nadine C. Fernandez, Caroline Flament, Florent Crépineau, Eric Angevin, Eric Vivier, Laurence Zitvogel
NK cells, dendritic cells, cell-to-cell interaction, activation, proliferation.

Altered dendritic cells (DC) might be responsible for regulatory T cell imbalance and autoimmunity in nonobese diabetic (NOD) mice

Sarah Boudaly, Joëlle Morin, Rolande Berthier, Patrice Marche, Christian Boitard
dendritic cell, nonobese diabetic mice, autoimmunity, insulin-dependent diabetes mellitus.

Estradiol and raloxifene decrease the formation of multinucleate cells in human bone marrow cultures

Ana Claudia Ramalho, Philippe Couttet, Claude Baudoin, Caroline Morieux, Anne-Marie Graulet, Marie-Christine de Vernejoul, Martine E. Cohen-Solal
osteoclast, estrogen receptor, SERM, cytokines, apoptosis.

Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal

Sara Gasperini, Luca Crepaldi, Federica Calzetti, Luana Gatto, Chiara Berlato, Flavia Bazzoni, Akihiko Yoshimura, Marco A. Cassatella
SOCS-3, PGE, IL-10, PBMC, neutrophils, PKA.

Different patterns of interleukin-1alpha and interleukin-1beta expression in organs of normal young and old mice

Moshe Hacham, Shmuel Argov, Rosalyn M. White, Shraga Segal, Ron N. Apte
IL-1alpha, IL-1beta, organ cytokine expression, young/old age, homeostasis.

The combination of the interleukin-1alpha (IL-1alpha-889) genotype and the interleukin-10 (IL-10 ATA) haplotype is associated with increased interleukin-10 (IL-10) plasma levels in healthy individuals

Sanna Kilpinen, Heini Huhtala, Mikko Hurme
interleukin-1, interleukin-10, gene polymorphism, plasma.

The interleukin-6 G(-174)C polymorphism and the ex vivo interleukin-6 response to endotoxin in severely injured

blunt trauma patients

Michael Heesen, Udo Obertacke, F. Ulrich Schade, Brunhilde Bloemeke, Matthias Majetschak
interleukin-6, endotoxin, genetic polymorphism, polymerase chain reaction, whole blood, critical illness.

Comparison of leptin- and interleukin-6-regulated expression of the rPAP gene family: evidence for differential co-regulatory signals

Daniël Broekaert, Sven Eyckerman, Delphine Lavens, Annick Verhee, Wim Waelput, Joël Vandekerckhove, Jan Tavernier
forskolin, IL-6, leptin, rPAP/Reg gene family, signal transduction.

Mice lacking alpha2-macroglobulin show an increased host defense against Gram-negative bacterial sepsis, but are more susceptible to endotoxic shock

Tino Hochepied, Fred Van Leuven, Claude Libert
TNF, shock, acute phase, inflammation, alpha2-macroglobulin.

Tumor necrosis factor-alpha mRNA stability in human peripheral blood cells after lipopolysaccharide stimulation

Lucile Baseggio, Carole Charlot, Jacques Bienvenu, Pascale Felman, Gilles Salles
tumor necrosis factor, mRNA stability, lipopolysaccharide, tristetraprolin.

Caspase-1 levels in biological fluids from patients with multiple sclerosis and from patients with other neurological and non-neurological diseases

Diego Franciotta, Gianvito Martino, Elisabetta Zardini, Roberto Furlan, Roberto Bergamaschi, Maira Gironi, Alessandra Bergami, Giovanna Angelini, Fabrizio De Benedetti, Patrizia Pignatti, Gianna Moscato, Vittorio Cosi
asthma, cerebrospinal fluid, juvenile idiopathic arthritis, sputum, synovial fluid, THP-1 cells.

Divergent effects of tumor necrosis factor-alpha and lymphotoxin-alpha on lethal endotoxemia and infection with live Salmonella typhimurium in mice

Edi Dharmana, Monique Keuter, Mihai G. Netea, Ineke C.M.M. Verschueren, Bart Jan Kullberg
tumor necrosis factor, lymphotoxin, salmonella typhimunium, knock-out mice.

Th1/Th2 cytokine balance and nitric oxide in cerebrospinal fluid and serum from patients with multiple sclerosis

María del Carmen Rodríguez-Sáinz, Silvia Sánchez-Ramón, Clara de Andrés, Margarita Rodríguez-Mahou, M Angeles Muñoz-Fernández
cytokine, nitric oxide, multiple sclerosis, cerebrospinal fluid, oligoclonal bands, Igs.

Retinoids, cisplatin and interferon-alpha in recurrent or metastatic cervical squamous cell carcinoma: clinical results of 2 phase II trials

Anne-Chantal Braud, Laurence Gonzague, François Bertucci, Dominique Genre, Jacques Camerlo, Gwenaelle Gravis, Anthony Goncalves, Vincent Moutardier, Frédéric Viret, Dominique Maraninchi, Patrice Viens
all-trans-retinoic acid, 13 Cis retinoic acid, interferon-alpha, cervical squamous cell carcinoma.

Circulating macrophage colony stimulating factor as a marker of tumour progression

Raymond S. McDermott, Laurent Deneux, Véronique Mosseri, Jacques Védrenne, Krishna Clough, Alain Fourquet, José Rodriguez, Jean-Marc Cosset, Xavier Sastre, Philippe Beuzeboc, Pierre Pouillart, Susy M. Scholl
CSF-1, tumour progression, metastasis, de-differentiation.

The effect of interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) on granulocyte macrophage-colony stimulating factor (GM-CSF) production by neuronal precursor cells

J. Benjamin Dame, Nasser Chegini, Robert D. Christensen, Sandra E. Juul
IL-1beta, TNF-alpha, GM-CSF, neuron, mRNA, protein.

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