



Cloning, expression and initial characterisation of interleukin-19 (IL-19), a novel homologue of human interleukin-10 (IL-10)

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Interleukin-10 (IL-10) is a pleiotropic cytokine with important immunoregulatory functions whose actions influence activities of many of the cell-types in the immune system. We report here identification and cloning of a gene and corresponding cDNAs encoding a novel homologue of IL-10, designated IL-19. IL-19 shares 21% amino acid identity with IL-10. The exon/intron structure of IL-19 is similar to that of the human IL-10 gene, comprising five exons and four introns within the coding region of the IL-19 cDNA. There are at least two distinct IL-19 mRNA species that differ in their 5'-sequences, suggesting the existence of an intron in the 5'-sequences of coding portion of the IL-19 gene. The longer 5'-sequence contains an alternative initiating ATG codon that is in-frame with the rest of the coding sequence. The expression of IL-19 mRNA can be induced in monocytes by LPS-treatment. The appearance of IL-19 mRNA in LPS-stimulated monocytes was slightly delayed compared to expression of IL-10 mRNA: significant levels of IL-10 mRNA were detectable at 2 h post-stimulation, whereas IL-19 mRNA was not detectable until 4 h. Treatment of monocytes with IL-4 or IL-13 did not induce de novo expression of IL-19, but these cytokines did potentiate IL-19 gene expression in LPS-stimulated monocytes. In addition, GM-CSF was capable of directly inducing IL-19 gene expression in monocytes. IL-19 does not bind or signal through the canonical IL-10 receptor complex, suggesting existence of an IL-19 specific receptor complex, the identity of which remains to be discovered. Genes and Immunity (2000) 1, 442–450.

Keywords: interleukin-10; interleukin-19; homologue; expression; monocyte; chromosome one; cloning

Introduction

Interleukin-10 (IL-10) is a multi-functional cytokine which is one of the key elements of the immune system.¹ Originally defined as 'cytokine synthesis inhibitory factor',² it is generally held to have anti-inflammatory actions through its ability to down-regulate antigen-presentation and macrophage activation in response to several stimuli.^{3,4} Awareness of this regulatory role has been strengthened by the discovery of regulatory T cells (Tr1-cells) demonstrating immunosuppressive functions both *in vitro* and *in vivo*, whose development and function depend on IL-10.^{5–7} In addition, a number of homologues of IL-10 exist in

human pathogenic viruses; the long-recognised Epstein-Barr virus (EBV) homologue^{8,9} was recently mirrored in the discovery of a cytomegalovirus (CMV) IL-10 homologue.¹⁰

IL-10 plays an important role in several human diseases. Its ability to promote B-cell activation and autoantibody production^{11,12} has led to the definition of possible roles for IL-10 in the pathophysiology of systemic lupus erythematosus (SLE) at both functional¹³ and genetic^{14–16} levels; similar mechanisms might apply to the involvement of IL-10 in rheumatoid arthritis (RA).^{17–19} In human malignant disease, IL-10 has a more ambiguous role. It has been shown that IL-10 suppresses T-cell responses in some malignant conditions²⁰ and that high expression of IL-10 can be observed in metastatic tissues.²¹ On the other hand, through its ability to inhibit the expression of matrix-metalloproteinases, IL-10 is capable of preventing the seeding of tumour cells into tissue²² and in this activity, as well as others²³ might therefore be said to give IL-10 an 'anti-metastatic' function. This activity may be mediated by IFN- γ ,²⁴ since the anti-tumour activity of IL-10 is lost in mice where the IFN- γ gene is deleted.

Many cytokines exist within structurally-related families. An example of this is the tumour necrosis factor

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(TNF) family of ligands which comprises three adjacent, closely-related genes within the MHC region on chromosome 6²⁵ and many other genes on a variety of chromosomes, which continue to be uncovered.^{26,27} More recently, the T-cell-derived cytokine IL-17 was shown to be the prototype for a new family of cytokines.²⁸ Given the wide range of activities of IL-10 and recognising that families of cytokines exist, we speculated that IL-10 too might be one member of a family of IL-10 related cytokines. In this report, we describe a human homologue of IL-10 and discuss the structure of its gene and protein in relation to IL-10 itself and other IL-10 homologues, and compare the expression of its mRNA with that of IL-10 in activated macrophages. We designated this new IL-10 homologue 'IL-19'.

Results

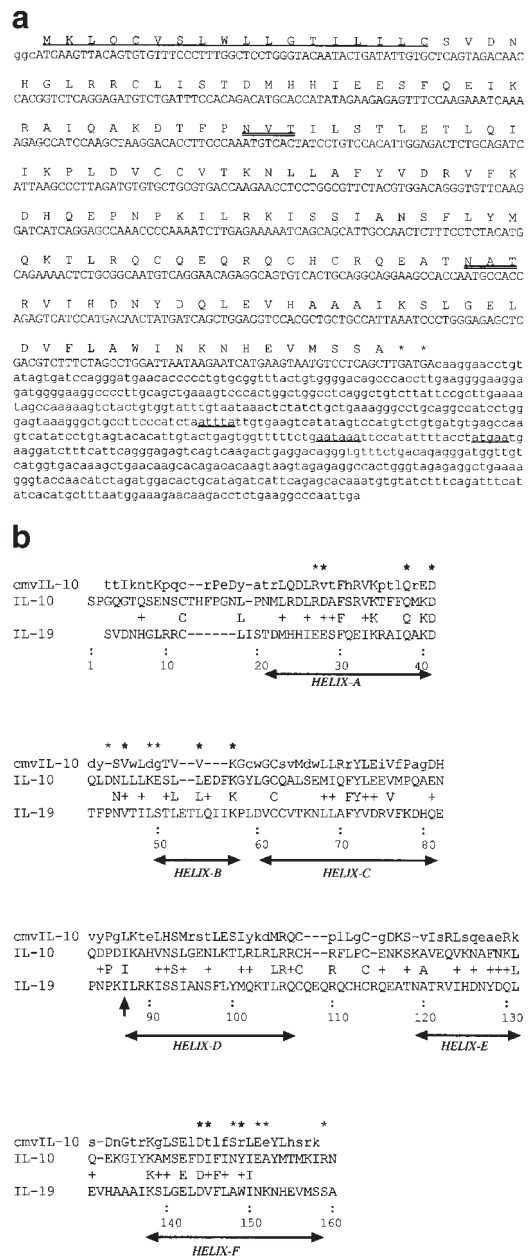
IL-19 is a novel human homologue of human IL-10

In two independent, parallel experiments utilising either the Genbank or TIGR databases, we identified the following ESTs as potentially encoding IL-10 homologues: AI266722, AA151656 and AA151652; the Genbank database also yielded ESTs AA151736 and AA151733. Following the screening of an EBV-transformed B-cell library, a full-length cDNA of approximately 1000 bp was obtained. The cloned cDNA (Figure 1a) encoded a protein of 177 amino acid residues. A hydrophathy plot of the protein suggested the presence of a signal peptide of about 18–20 amino acids at the N-terminus of the IL-10 homologue.

Alignment of the amino acid sequence of the protein, which we designated IL-19, with those of human IL-10 and cmvIL-10 (Figure 1b) revealed common structural features. The BLAST similarity between IL-10 and IL-19 is shown between the two sequences, while the recently-described cmvIL-10 is shown above (uppercase where

homologous or similar, lower case otherwise. Note that gaps are introduced in the cmvIL-10 to allow for the principle analysis, IL-19 vs IL-10). The four cysteine residues necessary to fold the IL-10 monomer correctly²⁹ are conserved (Figure 1b) and very high amino-acid matching is present towards the C-terminus. In addition, there is excellent conservation of the length of all IL-10 helices and inter-helix regions in the IL-19 molecule, suggesting that it adopts a similar folded structure to that of IL-10. Furthermore, 82% of the 50 amino-acids previously determined to be components of the hydrophobic core²⁹ which produces the homodimeric secreted form of IL-10 are conserved or are similar in the IL-19 molecule and so IL-19 may well have a similar, homodimeric, secreted form. However, of the 17 amino acids which are thought to interact with the IL-10 receptor A chain (10 in helix A/B and seven in helix F³⁰) which were predicted to

Figure 1 (a) cDNA and predicted amino acid sequence of human IL-19. Human IL-19 cDNA was cloned and sequences as described in the text. The predicted amino acid sequence reveals an 18-aa leader sequence (underlined) and a predicted mature form of 159 amino acids. Translation stop codons are indicated with a star (*). Two putative glycosylation sequences are indicated (double-underlined), as are a single mRNA instability motif (ATTTA), the poly-adenylation signal (AATAAA) and the polyadenylation site (ATGAA), in the 3-UTR (underlined). (b) Comparison of IL-19 with IL-10 and cmvIL-10. The human mature IL-19 peptide was BLAST-searched for areas of identity and/or homology with human IL-10. Identical amino acids are shown and similar amino acids are indicated (+). Amino acids are numbered according to the mature form of human IL-10.^{1,29} The areas forming the helices of human IL-10 are shown and a strong similarity of IL-19 is apparent. Residues previously determined to be important for the interaction of human IL-10 with the IL-10R1 receptor chain³⁰ are illustrated with a star (*); those residues identical or similar in human IL-19 are shown with a bold star (**). For comparison, the peptide sequence of cmvIL-10 is also shown. This molecule has been demonstrated to signal through the human IL-10 receptor.¹⁰ Amino acids in cmvIL-10 which are identical or similar to those of human IL-10 are shown in upper case. It will be seen that cmvIL-10 shares greater homology with human IL-10 in those areas of interaction with the IL-10R1 receptor chain than does human IL-19. The functionally-relevant amino acid Ile87 is indicated with an arrowhead. Although this is conserved in human IL-19, this area of IL-19 is less closely-related to human IL-10 than is cmvIL-10, supporting the observation that IL-19 does not signal through the canonical IL-10 receptor complex.



interact with IL-10R1 (the ligand-binding first chain of the IL-10 receptor complex), only nine are conserved or similar in IL-19 and so this molecule probably does not interact with the IL-10 receptor.

Structure of the human IL-19 gene

The coding region of the IL-19 mRNA is contained within a 5943 bp genomic fragment (Figure 2a), which compares with 3747 bp for human IL-10. Its structure consists of five exons and four introns, identical to that of IL-10. The intron/exon boundaries conform to the GT/AG rule (Figure 2b). The lengths of the respective exons are virtually identical between IL-19 and IL-10 (144 bp, 66 bp, 153 bp, 75 bp and 96 bp for IL-19 compared with 165 bp, 60 bp, 153 bp, 66 bp and 93 bp for IL-10; exons 1–5, respectively), although the intron lengths vary. The 3'UTR of IL-19 is much shorter than that of IL-10 (354 bp *vs* 1033 bp, respectively) and contains only one 'ATTTA' mRNA destabilising segment, compared with the five such segments found in the IL-10 mRNA (Saccini, SV Kotenko and S Pestka, unpublished results). The genomic IL-19 sequence is deposited with GenEMBL under accession number AF276915.

IL-19 mRNA has a complex 5' untranslated region

We have sequenced IL-19 cDNA clones from several sources, including stimulated peripheral blood mononuclear cells and an EBV-transformed B-cell library; the longest cDNA sequences obtained are reported here. In addition, genomic sequence 5' of the ATG coding for the methionine present in both the cDNA clones was obtained from genome walking experiments. Interestingly, these experiments yielded three distinct sequences,

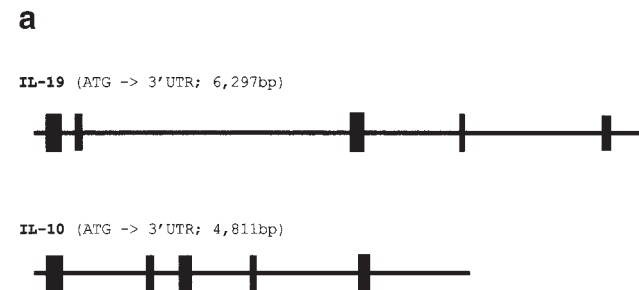
as shown in Figure 3. Two cDNAs have different 5'-sequences, neither of which is contiguous with the genomic segment which describes the first translated sequence. The sequences diverge starting two nucleotides – GC – upstream from the ATG codon. The genomic sequence has a consensus 3' splice acceptor site motif '(C/T)n.N.(C/A).A.G' – adjacent to these two nucleotides, indicating that there could be an intron close to the ATG codon. These data suggest that the human IL-19 gene can be alternatively-spliced upstream of this specific ATG codon, or alternatively be transcribed from two different promoters. The longer 5' sequence (Figure 3) contains a potential alternative translation site (double-underlined), in-frame with the main peptide sequence. The effect of beginning translation with this additional methionine is to add the following sequence to the amino-terminus of the translation product shown in Figure 1:

MCTEGAFPHRSACSLPLTHVHTHIHVCVPVLWGSVPRG

Such elongated leader sequences can modulate the secretion of a protein and have been observed in other cytokines, such as IL-15.³¹

Regulation of human IL-19 mRNA in human monocytes

IL-10 gene expression can be induced by a variety of stimuli, including LPS, in human monocytes.² The appearance of IL-10 mRNA in LPS-stimulated monocytes



b

Exon - Intron boundaries of the human IL-19 gene

Exon (donor site)	Intron	Exon (acceptor site)
1 AAGGCCATC	GTGAGTATGG . . . CCTCCACAG	2 CAAGCTAAGG
2 GATCATTAAAG	GTATTGGCCT . . . GTGTTTGTAG	3 CCCCTAGATG
3 GCGCCAATGT	GTGAGTCACT . . . TTTATCACAG	4 CAGGAACAGA
4 CTATGATCAG	GTAAGATCTG . . . CTGATTCAG	5 CTGGAGTCC

Figure 2 Genomic structure of IL-19. The genomic structure of human IL-19 was compared with that of human IL-10. Both genes comprise similar numbers of exons and introns, although the overall length of the IL-19 gene is greater. The sizes of the various exons are very highly conserved between the two genes, although the 3'-UTR of IL-10 is longer (a). The junctions between the exons and introns of human IL-19 conform to standard motifs (b).

Genomic

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ACTCATATCC AGTTGACTCA AGAAAACCAG AGGAGATACA TACCCCTGTA -151
ACTCCTCAAG GCTGAAGTGT TTATGTGTG TTTTCTTGTA TTCTCCCGG -101
CCTGCCTTCG AGGCTGGAAA GGAGCGTCCA ATCAGGGGTC TTCCTTTTCA -51
TTGCCCTCCA CTCTTGGCTG GACAGCTGAC TTCTCTCTCT TTCGTCAGGC -1
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M K L Q...
ATGAAGTTACAG...

Long-form 5'UTR

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M C T E G A F P H R S
TGCCACACTGACAGGAGTCCAAGAATGTGCACTGAGGGGCGCTTTCGCCACAGATCT
A C S L P L T H V H T H I H V C V P V
CGGTGTTCTTACCACACTCACACATGTGCACACACATATCCATGTGTGTGCCAGTG
L W G S V P R G M K L Q...
CTTTGGGCTCTGTCCACGGGGCATGAAGTTACAG...
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Short-form 5'UTR

M K L Q...
ACTGAGAGGAGACACAAGGAGCAGCCCGCAAGCACCACCAACTGAGAGCATGAAGTTACAG...

Figure 3 IL-19 mRNA has a complex 5'UTR. Sequencing of the human IL-19 genomic segment upstream of the underlined ATG codon (ATG) 5'-UTR on the human IL-19 mRNA revealed three separate sequences. Two hundred bases of the genomic sequence 5' of the underlined AGT codon is shown, as is sequence coding for the first four amino acids. It reveals a consensus intronic 3' acceptor splice site two nucleotides 5' of the ATG. Two alternative sequences from the underlined ATG codon, from different IL-19 mRNAs are shown. The longer form contains an alternative translation start site (ATG, double underlined) which is in-frame with the rest of the IL-19 mRNA, leading to the production of an elongated leader sequence as shown. The shorter form does not.

is delayed relative to that of other LPS-inducible genes such as TNF- α and IL-1.³² Since IL-19 expression was induced following LPS-stimulation of unseparated PBMC, it is important to understand its control in purified human monocytes. To determine if expression of IL-19 mRNA in LPS-stimulated monocytes coincides with expression of IL-10 mRNA, we incubated purified monocytes with medium alone or LPS (100 ng/ml) for 0 to 4 h. At designated time points, the cells were harvested, RNA was isolated, and the levels of IL-19 and IL-10 mRNA were measured by Northern blotting. As shown in Figure 4a, LPS stimulation induced expression of IL-19 mRNA which was not detectable until 4 h post-stimulation. Although significant levels of IL-10 mRNA were detectable at 2 h post-stimulation, IL-19 mRNA was not detectable until 4 h post-treatment.

The level of IL-19 gene expression induced by treatment of monocytes with LPS alone was relatively low. To determine if costimulating monocytes with certain cytokines together with LPS results in increased IL-19 gene expression, we preincubated monocytes with medium alone (control), IFN- γ , IL-4, or IL-13 for 2 h. We then added LPS and incubated the cells for an additional 3 h. At the end of the second incubation period, the cells were harvested, RNA was isolated, and the levels of IL-19 and IL-10 mRNA were examined by Northern blotting. As shown in Figure 4b, priming monocytes with IL-4 or IL-13 but not IFN- γ , significantly increased the levels of IL-19 mRNA induced by subsequent stimulation with LPS. Although IFN- γ did not amplify IL-19 gene expression in LPS-stimulated monocytes, it did potentiate IL-10 gene expression.

To determine if treatment of monocytes with certain cytokines can induce IL-19 gene expression, we treated monocytes for 4 h with a panel of cytokines that included IFN- β , IFN- γ , IL-4, IL-10, IL-13, and GM-CSF, and then measured induction of IL-19 mRNA expression by Northern blotting. As shown in Figure 4c, only GM-CSF was able to induce IL-19 gene expression in monocytes. Therefore, although IL-4 and IL-13 can potentiate expression of IL-19 (and IL-10) when cells are costimulated with LPS, these cytokines (IL-4 and IL-13) do not by themselves induce IL-19 gene expression in monocytes. IL-19 mRNA was not detected in stimulated human T-cells (data not shown).

IL-19 is a secreted protein

Since the presence of a signal peptide was predicted, we determined whether IL-19 was secreted and the size of the secreted IL-19. Both constructs (FL-IL-19 and IL-19-FL) were able to drive the expression of the modified IL-19 derivatives. Both proteins were secreted and had an identical pattern on Western blotting with anti-FLAG antibody producing multiple bands in the region of 35–40 kDa (Figure 5). The fact that the sizes of both proteins were identical indicates that the prediction of the beginning of the mature polypeptide was approximately close to the native cleavage site. In addition, the appearance of multiple bands on Western blotting suggested possible glycosylation of the protein. Indeed, there are two potential sites for N-linked glycosylation, Asn56-Val-Thr58 and Asn135-Ala-Thr137 (Figure 1b). The treatment of the conditioned media with Peptide: N-glycosidase F resulted in the disappearance of the bands in the region of 35–40 kDa and the formation of a strong single band in the region

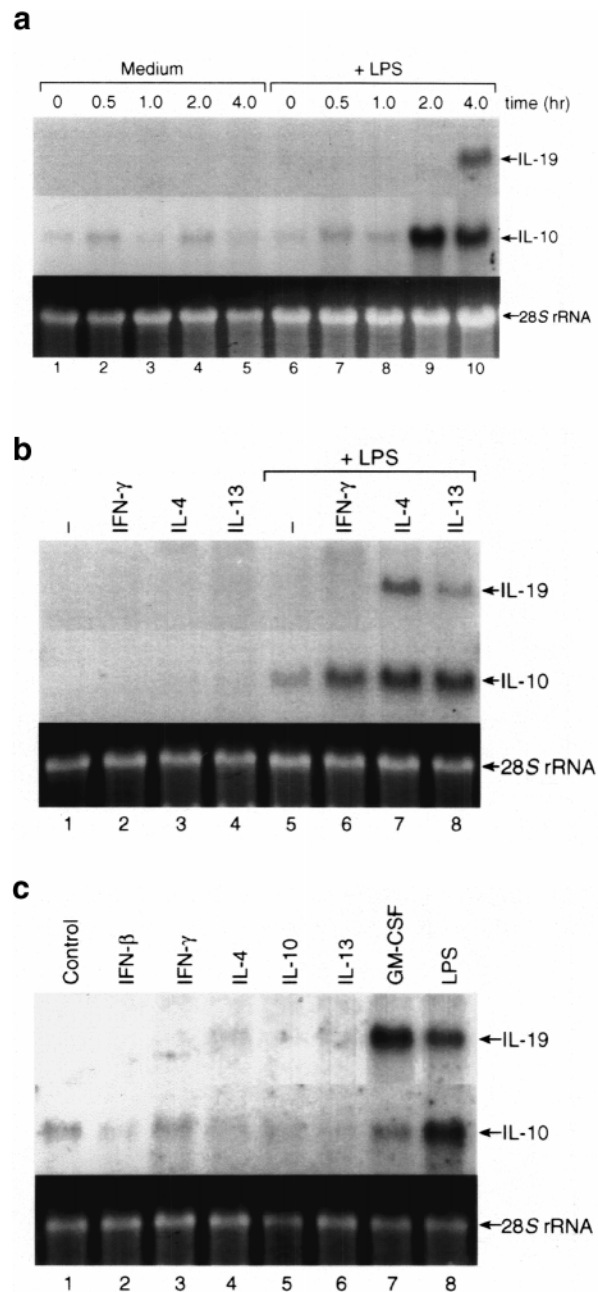


Figure 4 Regulation of IL-19 gene expression in purified human monocytes. (a) Effect of stimulation with LPS on IL-19 gene expression in monocytes. Monocytes (5×10^6 cells/ml) were incubated with either medium alone or LPS (100 ng/ml) for 0, 0.5, 1, 2, or 4 h at 37°C. At the indicated time points, RNA extracts were prepared, and the levels of IL-19 and IL-10 mRNA were measured by Northern blotting. (b) Effect of costimulation with LPS plus IFN- γ , IL-4 or IL-13 on IL-19 gene expression. Monocytes (5×10^6 cells/ml) were incubated with medium alone, IFN- γ , IL-4, or IL-13, in the presence or absence of LPS (100 ng/ml). After incubation for 3 h at 37°C, the cells were harvested, RNA was isolated, and expression of IL-19 and IL-10 mRNA was evaluated by Northern blotting. (c) Ability of selected cytokines to induce IL-19 gene expression in monocytes. Monocytes (5×10^6 cells/ml) were treated with either medium alone (Control), IFN- β , IFN- γ , IL-4, IL-10, IL-13, GM-CSF, or LPS for 4 h at 37°C. Each recombinant cytokine was added to monocyte cultures at a final concentration of 10 ng/ml. At the end of the incubation period, RNA was isolated, and expression of IL-19 and IL-10 mRNA was analysed by Northern blotting.

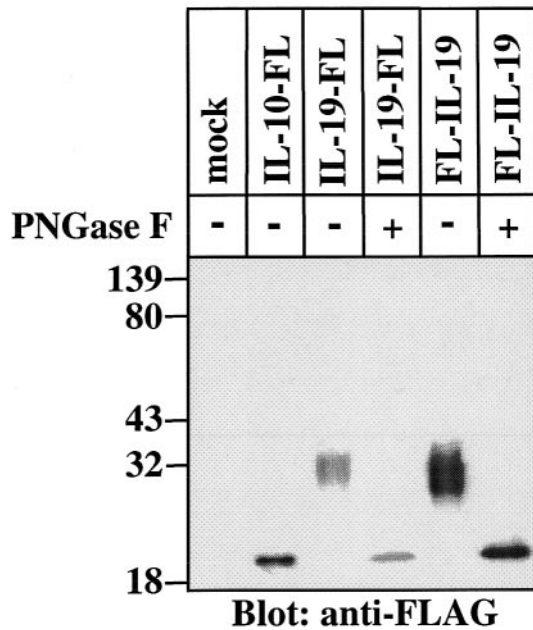


Figure 5 IL-19 expression. Western blotting analysis of COS-1 cells conditioned media. COS-1 cells were transiently transfected with the pEF-SPFL (lane 1, mock), the pEF-IL-10-FL (lane 2, IL-10-FL), the pEF-IL-19-FL (lanes 3 and 4, IL-19-FL), the pEF-SPFL-IL-19 (lanes 5 and 6, FL-IL-19) expression vectors. Three days later 10 μ l of the conditioned media were directly subjected to SDS-PAGE (lanes 1, 2, 3 and 5) or after being treated with P: N-glycosidase F (lanes 4 and 6). Western blotting was performed with anti-FLAG antibody. The molecular weight markers are shown on the left.

of 21 kDa for both proteins FL-IL-19 and IL-19-FL. The sizes of the deglycosylated proteins are consistent with the expected sizes and with the size of IL-10-FL (Figure 5). Interestingly, constructing the IL-19-FL expression vector using the longer 5'-UTR, encoding the extended signal peptide (Figure 3), severely limited the amount of IL-19 secreted.

Discussion

We have isolated and characterised a novel cDNA whose encoded protein is identified as a homologue of human interleukin-10. This cDNA is contained within a gene whose exon/intron structure is similar to that of the human IL-10 gene. The cDNA is expressed in human macrophages following LPS stimulation and this expression is regulated by cytokines that are known to modulate the expression of IL-10. No evidence for strong IL-19 expression in activated T-cells was observed. The new protein is capable of being secreted and glycosylated during transient production in transfected COS cells. We have termed this new gene 'IL-19'.

There is marked similarity between IL-19 and IL-10. The two proteins are of similar length (159 and 160 amino acids respectively, with additional leader sequences of 18 amino acids in each case). They appear to conform to an identical six-helix structure, each stabilised by two disulphide bonds whose positions are exactly conserved. IL-19 appears to have an identical hydrophobic core as IL-10 and when considered with the other structural similarities this suggests that it too is stable as a homodimer. Interestingly, those amino-acids present in the

hydrophobic core region of IL-19 which are not homologous to those in IL-10, correspond to those in positions previously defined to take no part in the hydrophobic interactions within the IL-10 molecule,²⁹ further emphasising the similarity between those which do match. Despite this strong structural similarity, IL-19 shows only moderate similarity to IL-10 in those regions corresponding to parts of the IL-10 molecule which were predicted to interact with IL-10R1.³⁰ Thus, IL-19 is unlikely to constitute an alternative ligand for the IL-10 receptor. This contrast is emphasised by a comparison with the recently-identified IL-10 homologue found in cytomegalovirus, cmvIL-10.¹⁰

The IFN- γ and IL-10 receptor complexes are structurally homologous.³³⁻³⁶ Although to date only the crystal structure of the complex between IFN- γ and its soluble IFN- γ R1 receptor has been solved,³⁷ based on the structural homology of the ligands and the receptor complexes^{29,30,37-39} a number of residues of IL-10 were proposed to be involved in interaction with the receptor.³⁰ The sequence of cytomegalovirus-encoded IL-10 (cmvIL-10) is included in the alignment because it provides additional information due to unique features of this protein. It was recently demonstrated that despite the low homology between IL-10 and cmvIL-10 (26% identity), cmvIL-10 binds and signals through the IL-10 receptor complex and competes for the receptor binding sites with IL-10.¹⁰ Thus, it is likely that the residues participating in interaction with the receptor should be conserved between IL-10 and cmvIL-10. Residues of IL-10 believed to be involved in interaction with the IL-10 receptor first chain are marked with asterisks and those which are conserved or similar in IL-19 are marked in bold asterisks. The alignment shows that a number of the residues for IL-10/receptor interaction which are not conserved in IL-19 are in fact conserved in the cmvIL-10 molecule, further indicating that IL-19 is unlikely to interact competently with the IL-10 receptor. Furthermore, IL-19 is less similar to IL-10 in the regions flanking the IL-10 residue Ile87 than is cmvIL-10. This amino-acid is known to be critical to the function of IL-10⁴⁰ and might interact with the IL-10 receptor second chain. Indeed, preliminary experiments have failed to demonstrate competition between IL-19 and IL-10 for the IL-10 receptor (SVK and SP, data not shown).

It is noteworthy that IL-19 cDNAs isolated from freshly-stimulated human PBMCs differed in their immediate 5'-sequences and that these, too, varied from the genomic sequence immediately upstream of the codon for the methionine present in both the cDNA clones. In the genomic sequence there is a 3' intron/exon splice-acceptor site two nucleotides upstream of the ATG codon, suggesting the possibility of an intron in the IL-19 5'-UTR. An examination of genomic sequence at the site of the 5' sequence of the shorter cDNA shows a consensus intronic 5' motif, exactly as required to permit splicing of this element to the coding exon. However, no evidence exists to suggest that the genomic DNA upstream of this shorter 5'-UTR could function as an inducible or constitutive promoter (not shown). The sequence encoding the most 5'-region of the longer cDNA is approximately 25 Kb further upstream in the genome from the sequence of the shorter cDNA. This also has a consensus 5' intronic motif which allows it to be spliced in-frame with the exon of the coding sequence. Beginning

translation from this additional ATG codon would lead to a longer leader sequence on the IL-19 protein (Figure 3) and preliminary evidence suggests that this form is not as readily secreted as that produced from the mRNA coding for the shorter form. Thus it appears that the IL-19 mRNA is subject to more complex processing than that of IL-10, where there are no known alternative translation start sites. This matter remains to be resolved but is very reminiscent to the situation seen with IL-15. Interleukin-15 was also reported to be expressed from two alternative promoters, one of which also generates a longer leader sequence. The shorter leader sequence is more poorly translated and furthermore appears unable to be secreted.⁴¹ The longer leader sequence drives post-translational modification producing several IL-15 isoforms, one of which is translocated to the nucleus and another of which is ultimately secreted (as reviewed in Waldmann and Tagaya³¹). The degree to which the regulation of many of these cytokines generally is so complex is not yet known. However, while many cytokines have uncomplicated promoter structures, it is becoming apparent that complex alternative splicing and promoter usages (including intronic promoters) may be frequent in molecules used by the immune system. Of particular note are the many alternative splice forms from the LST-1 gene in the TNF cluster,⁴² the use of alternative promoters in the oncostatin-M gene⁴³ and the CCR5 gene.⁴⁴ Indeed, the complex genetic nature of the human IL-10 promoter and its role in IL-10 secretion, may be an other reflection of this phenomenon.⁴⁵⁻⁴⁷ The observation that neither of the IL-19 mRNA 5' elements was contiguous with the genomic segment for the first coding exon suggests that IL-19 may also have a complicated regulation, perhaps varying in different cell-types.

The appearance of IL-19 mRNA in LPS-stimulated monocytes coincided with expression of IL-10 mRNA. Unlike other LPS-inducible cytokine genes such as TNF- α and IL-1, which are expressed within 1 h post-stimulation (not shown), mRNA for the IL-19 and IL-10 genes is not detectable until 2-3 h post-stimulation. This delayed induction of IL-19 and IL-10 gene expression is consistent with a role for these cytokines as feedback inhibitors of proinflammatory cytokine production. Previous studies have shown that blockade of IL-10 activity with neutralising anti-IL-10 Abs markedly increases TNF gene expression and TNF production.^{3,32} The fact that inhibition of TNF expression cannot be completely reversed by anti-IL-10 Abs indicates that other IL-10-like cytokines may be produced by LPS-stimulated monocytes.

Although treatment of monocytes with IL-4 and IL-13 alone did not induce *de novo* expression of IL-19, these cytokines did potentiate IL-19 gene expression in LPS-stimulated monocytes. Priming monocytes with IL-4 or IL-13 also amplified expression of IL-10 mRNA in LPS-stimulated monocytes. These findings are consistent with a recent report,⁴⁸ which demonstrated that IL-4 and IL-13 can increase IL-10 production by LPS-stimulated murine macrophages. IL-4 and IL-13 inhibit production of proinflammatory cytokines such as TNF and IL-1 by monocytes.^{3,49,50} Our finding that IL-4 and IL-13 amplify IL-10 and IL-19 gene expression in cultures of LPS-stimulated monocytes may explain how IL-4 and IL-13 inhibit production of cytokines such as TNF and IL-1. Although most of the cytokines that we tested did not induce IL-19 gene expression unless the cells were costimulated

with LPS, GM-CSF was capable of directly inducing IL-19 gene expression in monocytes. These findings are compatible with a related study by Lehmann *et al*,⁵¹ which demonstrated that GM-CSF can induce IL-10 expression in the monocytic cell line U937.

The presence of this new cytokine, a homologue of IL-10, raises questions about the complexity of immunosuppression which will only be answered by further experimentation. The initial screens for IL-19 revealed ESTs which were obtained from pregnant uterine cDNA and we cloned the mRNA from EBV transformed B-cells. It is known that during pregnancy immunity of the mother is shifted toward Th2 type of immune response,^{52,53} IL-19 may be the cytokine contributing to this shift. In addition, we have cloned the IL-19 cDNA from EBV-transformed lymphocytes, suggesting that IL-19 could be expressed by EBV-infected cells. It is known that both EBV-encoded and host IL-10s are expressed by virus infected cells. Expression of IL-19 could therefore contribute to the local suppression of the immune response mediated by IL-10-type immunosuppressive molecules. Furthermore, during the preparation of this manuscript we became aware of two independent reports also identifying IL-10 homologues. A protein designated 'IL-TIF' is expressed by IL-9 treated murine T-cells⁵⁴ and an IL-10 homologue designated 'AK-155' was cloned as a protein expressed by herpesvirus saimiri-transformed T-lymphocytes.⁵⁵ Thus, the identification of IL-19 extends the family of IL-10-related proteins, bringing the number of family members to four.

Our preliminary data suggest that IL-19 does not utilise the IL-10 receptor complex for signaling. However, it is possible that it may share one of the subunits of the IL-10 receptor complex, particularly IL-10R2, requiring distinct, ligand-specific additional receptor chains. A number of orphan receptors from the class-II cytokine receptor family (to which both subunits of the IL-10 receptor belong) have been identified³⁵ and are strong receptor candidates for these new cytokines. Thus IL-10, like so many other cytokines, exists within the framework of a family of cytokines which may have related, but subtly distinct, activities.

Materials and methods

Cloning of the IL-19 mRNA

In parallel experiments, the Genbank and TIGR databases were interrogated for sequences similar to IL-10. Each of the identified cDNAs encoded part of the c-terminus of a protein and the 3' untranslated portion of its cDNA. The contig of these ESTs was used to design primers for cloning a full-length IL-10 homologue cDNA by nested PCR from a library containing cDNA from EBV transformed human B-lymphocytes (Clonetech, catalogue number HL4006AE). Specific primers:

IL-19cDNA.1 5'-CTT.CAA.GGT.GGG.CTG.TCT.CC 3'
IL-19cDNA.2

5'-GTT.TCT.AGA.ATT.CAA.GCT.GA.GAC.ATT.ACT.TC 3'

were used in conjunction with vectorprimers:

5'-CTA.TTC.GAT.GAT.GAA.GA.TAC 3'

and

5'-ATA.CCC.CAC.CAA.ACC.CAA 3'

The resulting cDNA was ligated into the pPCR 2.1 vector (Invitrogen) and sequenced.

Sequencing and characterisation the IL-19 gene

The coding portion of the IL-19 cDNA was used to design primers for the cloning of the IL-19 gene.

IL-19genF:
5'-ATG.AAG.TTA.CAG.TGT.GTT.TCC.CTT 3'
IL-19genR:
5'-TCA.AGC.TGA.GGA.CAT.TAC.TTC.ATG 3'

250 ng of human total genomic DNA was amplified using these primers in association with the 'Extensor Long' PCR system from Advanced Biotechnologies (England) following the manufacturer's protocol. Electrophoresis on a 2% agarose gel (Gibco) revealed the presence of a single band of approximately 6 kb. This genomic fragment was purified (Qiagen) and forwarded to the Sanger Centre for sequencing (Cambridge, England; Simon Gregory, head of chromosome 1 team). Intron/Exon boundaries were assigned following alignment of the genomic and mRNA sequences. Confirmation of the 3' UTR region of the cDNA and additional 5' and 3' genomic sequence was obtained using the 'Genome Walker Kit' (Clontech). Specific and nested primers were designed from the coding sequence and used with the kit reagents as supplied and according to the manufacturer's recommendations.

Expression of IL-19 mRNA in activated macrophages by Northern blot

An IL-19 probe was prepared by gel-purification of the PCR product obtained by amplifying LPS-stimulated human PBMC cDNA using the following primers:

IL-19mF: 5'-ATG.CAC.CAT.ATA.GAA.GAG.AGT 3'
IL-19mR: 5'-CAG.CTG.ATC.ATA.GTT.GTC.ATG 3'

under the following conditions: denaturation at 94°C for 4 min followed by 35 cycles of 94°C, 1 min; 55°C 1 min; 72°C, 1 min; and completed with a final extension step at 72°C for 10 min. This probe was radiolabeled by the random primer method⁵⁶ to a specific activity of 10⁸ cpm/μg or greater.

Normal human peripheral blood monocytes were isolated by countercurrent centrifugal elutriation in a Beckman JE-6B centrifugal elutriator (Beckman Instruments, Inc., Fullerton, CA, USA) as previously described.⁵⁷ The elutriated monocyte fraction consisted of >95% monocytes as determined by histologic staining and FACS analysis after labelling with the anti-CD14 mAb, Leu M3 (Becton Dickinson, Mountain View, CA, USA). Monocytes were routinely cultured at 4 × 10⁶ cells/ml in complete medium in round-bottom, polypropylene tubes (17 × 100 mm).

Monocytes were cultured in RPMI-1640 medium (GIBCO-BRL, Gaithersburg, MD, USA) supplemented with 10% (v/v) heat-inactivated foetal calf serum (Hyclone, Logan, UT, USA), 2 mM L-glutamine, and 50 mg/ml gentamycin (Complete Medium). Recombinant human IL-4 and rhIL-10 were provided by Schering-Plough, Inc (Kenilworth NJ, USA). Recombinant human IL-13 was obtained from BioSource International (Camarillo, CA, USA). Recombinant human IFN-γ was provided by Genentech, Inc (So. San Francisco, CA, USA). Recombinant human IFN-β was provided by Chiron Corp (Emeryville, CA, USA).

Total RNA was isolated from cultured monocytes by the acid guanidinium thiocyanate-phenol-chloroform extraction method as previously described.⁵⁸ RNA precipitates were pelleted by microcentrifugation, and redissolved in 100 μL of DEPC-treated sterile water. After quantitation by spectrophotometry, equivalent amounts of RNA (10 μg/lane) were size-fractionated by electrophoresis in 1% agarose gels containing 0.66 M formaldehyde. The RNA was then blotted by overnight capillary transfer onto Nytran membranes (Schleicher & Schuell, Keene, NH, USA), and crosslinked by exposure to UV light. The membranes were then prehybridized, hybridized and washed according to standard procedures.

Transient expression of the IL-19 peptide

Two expression plasmids were constructed to determine whether IL-19 was secreted.^{33,59,60} One plasmid was designed to produce IL-19 tagged with the FLAG epitope at the N' terminus and the other to tag IL-19 at the C-terminus. In the first construct the fragment encoding the signal peptide of the human IFN-γ receptor 2 (IFN-γR2) followed by the FLAG epitope was fused with the fragment encoding the mature IL-19 starting after the predicted signal peptide at Ser19.

Primers
5'-GCC.GAT.CCC.TCA.GTA.GAC.AAC.CAC.GG 3'
and
5'-GTT.TCT.AGA.ATT.CAA.GCT.GAG.GAC.ATT.ACT.TC 3'

and the IL-19 cDNA were used for PCR to construct FL-IL-19. The PCR product was cloned into plasmid pEF-SPFL¹⁰ with the use of BamHI and XbaI restriction endonucleases. To create IL-19-FL, PCR was performed with primers:

5'-CTC.GGT.ACC.ACG.GTG.AAT.GAA.GTT.ACA.GTG.TG 3'
and
5'-GGT.GCT.AGC.TCG.TCG.AGC.TGA.GGA.CAT.TAC.TTC 3'

and the IL-19 cDNA. The PCR product was digested with KpnI and NheI restriction endonucleases and cloned into appropriate sites of plasmid pEF-X-FL. This plasmid was created by cloning the PCR product, obtained with primers:

5'-TCC.AAT.TCG.CTA.GCC.CTG.ACT.ACA.AGG.ACG.ACG 3'
and
5'-GTG.TCT.AGA.GCT.TGT.CAT.CGT.CGT.CCT.T 3'

and the pFL-CRF plasmid DNA as a template,³³ into EcoRI and XbaI sites of the pcDEF3 plasmid.⁶⁰ Thus, in the second construct the FLAG epitope was linked to the C-terminus of IL-19 retaining its own signal peptide. Both plasmids were transiently transfected into COS-1⁶¹⁻⁶³ cells and 3 days after transfection the conditioned media were subjected to Western blotting with anti-FLAG antibody. Both constructs were able to drive the expression of the modified IL-19 derivatives.

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