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Mapping of the human *IL10* gene and further characterization of the 5' flanking sequence

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Abstract Interleukin-10 (IL-10) is an important regulatory cytokine whose involvement extends into diverse areas of the human immune system. Recent characterization of the promoter and 5' flanking regions has demonstrated the presence of positive and negative regulatory segments in the promoter. In addition, the characterization of two dinucleotide repeat elements immediately upstream of the gene has shown that there is considerable polymorphism directly associated with the human *IL10* gene. In the present report, we describe the mapping of the human *IL10* gene to a discrete area of chromosome 1, the definition of a potential cytokine-responsive segment 3–4 kilobases upstream of the transcription initiation site, and the identification of two new point mutations in the immediate promoter region with their distribution in a panel of 75 unrelated healthy individuals. These data should further the understanding of how the *IL10* gene is controlled in humans and how its function may vary between individuals.

Introduction

The recent characterization of interleukin-10 (IL-10) as an important multifunctional cytokine has led to the recognition that it is a key component of many aspects of the immune response (Moore 1993; Mossmann 1994). The influence of IL-10 in the basic biology of the human immune system is reflected in its involvement with a range of malignant and autoimmune disease states.

The nucleotide sequence data reported in this paper have been submitted to the GenBank/EMBL nucleotide sequence databases and have been assigned the accession number (X78437)

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Interleukin-10 (IL-10) appears to have considerable importance in the development of human cancer. IL-10 is found at elevated levels in the serum of patients with common tumors (Dummer et al. 1995; Fortis et al. 1995) and this may be derived directly from the tumor cells; IL-10 protein or mRNA appear to be important components of the tumor microenvironment in a range of human cancer types, including renal cell carcinoma (Nakagomi et al. 1995) and skin tumors (Lüscher et al. 1994; Kim et al. 1995). In addition, IL-10 has been implicated in the development of tumor-associated anergy (Lüscher et al. 1994); this may involve the ability of IL-10 to reduce major histocompatibility complex (MHC) class II expression on macrophages (Malefyt et al. 1991a) and thus competent interaction with T cells (Fiorentino et al. 1991). Indeed, a recent report demonstrates clearly that IL-10 can maintain T-cell clonal anergy in the face of stimulation with MHC class II-positive human melanoma tumor-cell targets (Becker et al. 1994), illustrating the importance of IL-10 in this process. IL-10 antagonizes human T-cell clonal expansion (Taga et al. 1993a) and has been shown to inhibit the development of antigen-specific cytotoxic T cells (Bejarano et al. 1992). Potential tumor targets are further protected from cytotoxic T-cells by IL-10 reducing tumor cell MHC class I expression (Matsuda et al. 1994).

IL-10 is also important in the aetiology of human B-cell lymphomas, where its expression can be profoundly dysregulated. The Epstein-Barr virus (EBV) genome encodes a gene with structural and functional similarity to human IL-10 (Moore et al. 1993) and infection of normal human B cells with EBV leads to immortalisation of these B cells and an accompanying IL-10 production (Rousset et al. 1992). While the relationship between EBV and the human *IL10* gene is poorly defined, it has been suggested that the *EBNA2* gene product transactivates the EBV latent membrane protein-1 (LMP-1), or other cellular genes, which then activate the production of human IL-10 (Kube et al. 1994; Wang et al. 1990; Nakagomi et al. 1994). Interestingly, infection and transformation of human B-cells by EBV induces the production of the human (rather than the viral) form of IL-10 by the infected cells (Burdin et al.

1993) and raised levels of human IL-10 have been observed in the serum of patients with non-Hodgkin's lymphoma (Blay et al. 1993) and in EBV-positive Hodgkin-Reed-Sternberg cells of Hodgkin's lymphoma (Herbst et al. 1996). Furthermore, the human Herpes virus-encoded IL-10 homologue is instrumental in the induction of T-cell non-responsiveness to infected cells and subsequent tumorigenesis (Suzuki et al. 1995), while the natural human IL-10 functions as an autocrine growth factor in HIV-related B-cell lymphoma (Masood et al. 1995). Indeed, recent data have demonstrated that a number of viruses can initiate cytokine expression. In addition to IL-10, IL-1 α and IL-12p40 have also been observed (Schols and de Clercq 1996; Fantuzzi et al. 1996; Mori and Prager 1996).

While more usually associated with the inhibition of T-cell function, under conditions of IL-2 deprivation IL-10 can inhibit apoptosis in human T cells (Taga et al. 1993b) and can support the growth of certain T-cell clones, particularly those bearing a gamma-delta T-cell receptor (Pawelec et al. 1995). It is also a potent activator of normal B cells, stimulating both proliferation and differentiation (Rousset et al. 1992) and raised IL-10 levels have been reported in several autoimmune states (Llorente et al. 1994). This may be important in chronic inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematosus, where IL-10 has recently been shown to promote the secretion of rheumatoid factor (Perez et al. 1995) and other autoantibodies (Llorente et al. 1995). In this context, we have recently observed an association between elements within or close to the human *IL10* locus and the presence of SLE (Eskdale et al. 1997).

The potential importance of IL-10 and the *IL10* gene locus in the epidemiology of malignant and autoimmune disease has stimulated us to investigate further the structure of the *IL10* gene and its relationship with putative mRNA species which may be nearby. Understanding in such areas may give insight concerning to the involvement of this important cytokine in the question of genetic predisposition in human disease.

Materials and methods

Sequencing the human *IL10* 5' flanking region

As a prelude to sequencing the 5' flanking region of the human *IL10* gene, a cosmid gene bank (Stratagene, Cambridge, England) from human placenta was screened for *IL10* using two different hybridization probes, as previously described (Kube et al. 1995). The nucleotide sequence was determined using the USB rapid sequencing kit (Amersham, Amersham UK). We have defined the DNA sequence up to position -4082 from the transcription start site of the human *IL10* gene (with the base immediately preceding the A of the ATG taken as position -1). This sequence has been deposited in the GenBank and EMBL databases as HSINTL10 with accession number X78437 (1996 update; D. Kube). Putative transcription factor binding sites were assigned following examination of the sequence with the SIGSCAN software (which is part of the GCG package), or the MatInspector/TRANSFAC software, which is available on the World Wide Web, at URL http://www.gsf.de/biodv/matinspector_help.html.

Mapping the human *IL10* gene to chromosome 1q

We used the two dinucleotide repeats which lie in the 5' flanking region of the human *IL10* gene to analyze the position of this gene on the Whitehead Institute Radiation Hybrid map of human chromosome 1. The theory of radiation hybrid mapping was recently described by Cox and co-workers (1990). Basically, the human genome is fragmented by irradiation, with the degree of fragmentation being dependant on the radiation dose. Chromosomal fragments are then randomly inserted into hamster cell lines where they often become integrated into the hamster genome, thereby creating radiation hybrids. Examining a panel of such hybrids by PCR for the area of interest generates a pattern of positive and negative hybrid clones conforming to the particular fragments of the human genome they have acquired. Computer analysis of these results allows the positioning of the test marker (in this case, the human *IL10* gene) in relation to known standards. The distance between the test marker and known standards is given in centi-Rays (cR). cR are analogous to centi-Morgans but are determined by the initial irradiating dose used to construct the panel, rather than cytological markers. The Genebridge-4 release of the radiation hybrid panel was generously made available to us by the Human Genome Mapping Project at the MRC centre in Cambridge, England. The panel was amplified using the *IL10.R* and *IL10.G* microsatellite primers, as previously described (Eskdale and Gallagher 1995; Eskdale et al. 1996). A confirmatory analysis was performed for each primer set; no discrepancies were observed. Each primer set amplified an identical selection from the hybrid panel, with the exception that the *IL10.G* primers gave a positive result with clone 4M5, while the *IL10.R* primers were negative, and the *IL10.R* primers gave a positive result with clones 4M4 and 4R1, while the *IL10.G* primers were negative. The results from the screening were entered into the STS mapping program at the Whitehead Institute's World Wide Web site (URL: <http://www-genome.wi.mit.edu/>) as a continuous vector of "1" or "0", depending on whether amplification was observed or not. Hybrids 4BB12, 4A5, 4BB10, 4U3, 4B9, 4O10, 4B2, 4R12, and 4K8 were not supplied by the HGMP/MRC and so results for these were entered in their appropriate positions in the vector as "2" (unknown). The full vectors entered were:

IL10.G:
0200202011221011000000001100000010000000110200000100010-
0200001001001201010000020110000011000
IL10.R:
0200202011221011000000001100000010000000110200000100010
0200001101001201010000020110000011000

Definition of two point mutations in the human *IL10* promoter region

We made use of the Pileup software (part of the GCG package) to analyze the human *IL10* promoter sequences deposited in the GenEMBL database. This analysis revealed that the area between bases -1002 and -516 contained three possible polymorphic bases. If these were truly polymorphic, then polymerase chain reaction (PCR) amplification of the area of interest, followed by digestion with the restriction endonucleases *Mae* III, *Hpa* II or *Rsa* I should reveal the polymorphic nature of nucleotides -854, -686, and -627, respectively. PCR primers were obtained for this purpose from Cruachem (Glasgow, Scotland):

IL-10.5 5' GAC.TCC.AGC.CAC.AGA.AGC.TTA.C 3'

IL-10.6 5' AGG.TCT.CTG.GGC.CCT.TAG.T 3'

which complemented bases -1002 to -981 and -534 to -516, respectively, allowing amplification of the region containing all three putative polymorphic bases. Amplification was carried out with an initial denaturation of 5 min at 95 °C followed by 35 cycles of 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min; the reaction was completed by a final extension step of 72 °C for 5 min. The final reaction volume was 75 μ l in all cases and reactions contained 1 unit of Primezyme thermostable polymerase (Biometra, Göttingen, Germany) in the presence of 1.5 mM magnesium as Optimal Buffer. Aliquots (10 μ l) of reaction product were digested in a final volume of 20 μ l with 1 unit of *Mae* III, or 5 units of *Hpa* II or *Rsa* I, according to the manufacturer's instructions (all from Boehringer Mannheim, Mann-

Fig. 1 (For continuation and legend see p. 123)

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AGATCTTGTA AACTGTAGAA TGCACCCCTCC AAAATCTATT TGCATAAGCA CACACACACA CACACACACA CACACCCCA CAGTCTCTGC -3993
CTGCCAGAT TCCTCTGCAG CTAAAGTGAT GAAACTTACT GGGCGGAGCT TCCTAAAAAG ATTATTAGGG TCTCCTGGGT TGGTGTGCCT -3903
TTAAACCTTT GGACTTTACC ACCTCCTATC TCTCCTATCT CCTTGCAACA AAGGTTAGGA GAACAAGAAT CGAGAAAAAA CGGGTCTCTGG -3813
ATGACATCTG AGTGCCTGCT TTGGGCTTCT TGATGAGTGA GACAGAAAAT AAAATACAAC CCCCTCTTTT AAAAGCCATG CTTACTCAGG -3723
TTTTCTTCA TTTGCAGCTA AATACAGAAA TGAGAGAATA TTTTGGAGCA GGGATGGAAG AAGAGAGGTA TTCCCTTCC CACAACCTTC -3633
TGATTTCCCA GTACATCCCC CACTGGAAAA ATTCAATTTAA AATCAGTATA ATAAGCATTG ATTAGATGCC TACTATGCAT CTGGGCTTGA -3543
GGCAAACCTG GACTCAGGCC TTTTGGCCTC AAGAAGCTCA CAGTGTGAGA GTGGCATTGG TGTCCTCTTG AAATTCACAG GACTAAATTC -3453
TGCCAGGCT GACATCTTAT CCATCCATAG GTGCCTGCCT TCTCACTTCC CTCTCTTCAT GGGCTCTTG CTGTACCAA AATCCAACC -3363
CAAATCTCCT CACATGTGAG TGTGGCATT CATGTCTCAG ACATGACCTA TGGGCTTGGG ACTTTTCCCC GTGGACCCA GTGACTTTTC -3273
AGATGAACAG GTATCTTCAA AAACCTGAGA AATAGGAGTC CTGTTTGTG TTCTTGTTC TTTGTCAATA TAAGGCACAG GGTCTTTTATT -3183
CAAATGTCA TACTATCTCT TGACAGAAAT ACTATGAGAC ATATTGATGG AGAAGCCGTT ATCTCCATAT GCTAAATGAG GACTTGCACC -3093
AGGGAACCTG CCCATGGTTC TCTCCAACCA CTTAAATTTCT GAAATTTTGA AATGAGAGTG GACAGTAATT TCAAATCAAT GGGGAAAGAA -3003

TCAAATCTTC AGCAAATGGC TTGAGATAAT TAGCTACACA TTTCAGAACA AATAAAGAAG TCAGATCCGG GCCGGGCACA GTGGCTCATG -2903
CCTGTAATCT CAGCACTCTG GGAGCCAAG GCGGGCGGAT CATAAGTCA GGAGATCGAG ACCATCTGG TTAACACAGT GAACCCCTCT -2813
CTAATAAAAA TACAAAAAAA AATAAAAAA CTTAGCCGGG CGTGGTGCCA GCGCCTGTGA GTCCACGTA CTCGGGACGC TGAGGCAGGA -2723
GAATGGCTTG AACTCGGGAG GCAGAGCTTG CAGGTGAGCT GAGATCATGC CACTGCACCT CAGCCTGGGC AACAGAGCGA GACTCTGTCT -2633
CAAAAAAAA AAAGAAGTCA GATCCTAACC TCAACCCTAT TTAACAGATT ATAGATGAAA GAAAGGTACA AATGGCTTTT ACATACCTCC -2543
CTTCTCCCTG ACATTTTSTA TGTGTGTGTG TGTGTAATTA CACACACATC TCATATAAGG AAATTTGAAG GAGGCTGCCT GCATCCCTGA -2453
GTCACTCTCC CTCTCTTCT GAATGCTTAC CTGTGCCAG ACCACCTCCT TAGCCTCGCA CCCTCCAGG TTACAGGGCA CTCTCTATG -2363
CCCATCCCAA GTATAGCTGA TACCTTCCAA GGGCCAGACT TGGTGCCTAAG TACCAAGTAC GCAAAGATTA ATAAACAAT GTCCGTGTTT -2273
AGGGAGCTCA AAGCTGATTC GGCAGGGCAT GGTGTGTACA TGAATGATAA CCACGTAGG TTGCAGGTTT CCTAGTGAGG TAAGCACAAG -2183
GCAAGATGGG AAACAAAGGA AGGAGGGGTT CACAGCCTCA CCCAGAGTCC AGAACCCCTG GCCTGCCTGG TGCCCATGCT GAGTCCACTT -2093
CTGGAACACC CAGCTCAGAG AGGGGGTTAG ACCTGCAGGC TAACACAGAC ACAGCCAGA AAACCCAGGA GCCGAGGGGG AAGGAGAAA -2003
GTGCAAGAAG GGGAAACCCA GTCTCTGGT CCCTTCTCTC TGCTTCTGG CAGCAGAACT CAGACAGAAC CCTTAAGCCA GTCTAAGTCT -1913

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heim, Germany). Digestion fragments were separated in 2% agarose (Gibco-BRL, Paisley, Scotland) in $1 \times$ TAE buffer and visualized by ethidium bromide staining. A 100 base pair (bp) ladder (Gibco-BRL) was used to calibrate the gels.

Results

Sequence of the human IL10 5' flanking region

The sequence of the human *IL10* gene from +131 to -4082 is shown in Figure 1. This sequence supercedes our original deposition and can be accessed from the GenBank/EMBL database under the accession number X78437. The Figure shows the sites of the two microsatellites *IL10.G* and *IL10.R* and a putative inverted repeat sequence beginning at -2522, the functional significance of which remains to

be determined. The *GM-CSF* palindromic element is also shown, at -2325.

Previously (Kube et al. 1995), we defined the location of transcription factor binding motifs in the immediate promoter of the *IL10* gene. In this report, we also examine the *IL10* 5' flanking sequence for the presence of potential transcription factor binding sites, paying particular attention to those thought to transduce signals from cytokines or other elements of the immune system; their locations are shown in Figure 2. The sites included the NF κ B/REL sites known to mediate tumor necrosis factor signalling (Hou et al. 1994; Ohmori and Hamilton 1995; Meyer et al. 1996) and the STAT.1 sites known to mediate interferon and interleukin signalling (David 1995; Ihle 1996). Also common were sites corresponding to the interferon-gamma sequence motif from *HLA-DR* genes (Yang et al. 1990), the NF-IL6 recognition sequence (Akira et al. 1990), and

Fig. 1 Sequence of the human *IL10* gene 5' flanking region. The positions of the *IL10.G* and *IL10.R* microsatellites are shown, as are the *IL10* inverted repeat and the *GM-CSF* palindromic sequence

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GGCAGGACCA GTAAGTTC TG AGTTAGCTCC ATACTAGTTT CTAGCAGGCT CTTTCTCACT TCCTGATPCT TAGGTTTCTA CATTGACACT -1823
CCCTGAAGAG TTGGGAAGAG ACACCACAGT CCCCTGACCC TGATCCATAG GTCACACAGC AGGCATCCAC AGGGTGGGCG TGGGCCCTCT -1733
CATCCCTCCC TCCCACCTCAC TTCACGCTGG CTGGGCCCCA AGGTGTTTGC ACCCCTTGCA GTGAGTGACC TTCTCTAGTG CAGCAAGCTC -1643
AGAACCTGCT GCCACTGGAG TTGTCCCATT GCTGATGCAG AAAGGTGAAG AACTAGCAGA AACTTGAAA TGCCCTCCAT CTGGGTCCAT -1553
GGCTACTTAA TGCTCCCTGG CAGGCAGGAG GACAGGTGCT ATTCCTGTGTT GGGACAGATG AAAAACAGAC ACAGGGGAGGA TGAGTGATTT -1463
GCCCTGACTA TAGAGTGCCA GGCCCAAGGC AGAGCCCAGG CCTCTGCAC CTAGGTCAGT GTTCTCCCA GTTACAGTCT AAACCTGGAAT -1353
GCAGGCAAAG CCCCTGTGGA AGGGGAAGGT GAAGGCTCAA TCAAAGGATC CCCAGAGACT TTCCAGATAT CTGAAGAAGT CCTGATGTCA -1263
CTGCCCCGGT CCTTCCCCAG GTAGAGCAAC ACTCCTCGCC GCAACCCAAC TGGCTCCCCT TACCTTCTAC ACACACACAC ACACACACAC -1173
IL10.G microsatellite
ACACACACAC ACACACACAC AAATCCAAGA CAACACTACT AAGGCTTCTT TGGGAAGGGG AAGTAGGGAT AGGTAAGAGG AAAGTAAGGG -1083
ACCTCCTATC CAGCCTCCAT GGAATCCTGA CTCTCTTTCC TTGTTATTTT AACTTCTTCC ACCCCATCTT TTAACCTTTA GACTCCAGCC -993
ACAGAAGCTT ACAACTAAAA GAAACTCTAA GGCCAAITTA ATCCAAGGTT TCATTTCTATG TGCTGGAGAT GGTGTACAGT AGGGTGAGGA -903
AACCAAATTC TCAGTTGGCA CTGGTGTACC CTTGTACAGG TGATGTAACA TCTCTGTGCC TCAGTTTGCT CACTATAAAA TAGAGACGGT -813

AGGGGTCATG GTGAGCACTA CCTGACTAGC ATATAAGAAG CTTTCAGCAA GTGCAGACTA CTCTTACCCA CTTCGCCCAA GCACAGTTGG -723
GGTGGGGGAC AGCTGAAGAG GTGGAACAT GTGCCTGAGA ATCCTAATGA AATCGGGGTA AAGGAGCCTG GAACACATCC TGTGACCCCG -633
CCTGTCTGTG AGGAAGCCAG TCTCTGAAA GTAAAAATGA AGGGCTGCTT GGGAACTTTG AGGATATTTA GCCCACCCCC TCATTTTATC -543
TTGGGGAAC TAAGGCCAG AGACCTAAGG TGACTGCCTA AGTTAGCAAG GAGAAGTCTT GGTATTCAT CCCAGGTTGG GGGGACCCAA -453
TTATTTCTCA ATCCCATGTG ATTCTGGAAT GGGCAATTTG TCCACGTCAC TGTGACCTAG GAACACGCGA ATGAGAACC ACAGCTGAGG -363
GCCTCTGCGC ACAGAACAGC TGTCTCCCC AGGAAATCAA CTTTTTTTAA TTGAGAAGCT AAAAATTTAT TCTAAGAGAG GTAGCCCATC -273
CTAAAAATAG CTGTAATGCA GAAGTTCATG TTCAACCAAT CATTTTGTCT TACGATGCAA AAATTGAAA CTAAGTTTAT TAGAGAGGTT -183
AGAGAAGGAG GAGCTCTAAG CAGAAAAAT CCTGTGCCGG GAAACCTTGA TTGTGGCTTT TTAATGAATG AAGAGGCCCT CCTGAGCTTA -93
CAATATAAAA GGGGACAGA GAGGTGAAGG TCTACACATC AGGGGCTTGC TCTTGCAAAA CCAAACACA AGACAGACTT GCAAAAAGAG -3
+1
GCATGCACAG CTCAGCACTG CTCTGTTGCC TGGTCCCTCT GACTGGGGTG AGGCCAGCC CAGGCCAGG CACCCAGTCT GAGAACAGCT +88
GCACCCACTT CCCAGGCAAC CTGCCTAACA TGCTTCGAGA TCT +131

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the GM-CSF consensus sequence (Nimer et al.1990). The palindromic repeat sequence associated with activation of the human *GM-CSF* gene and found in several cytokine genes (Staynov et al. 1995) was also observed, as were the CD28-like (Curtiss et al. 1996), the GAS-like (Galsgaard et al. 1996), the GAS-LKRE (Tsukada et al. 1996), and the YY1-like (Yant et al. 1995) factor recognition sequences, described by others as being involved in the control of immune and inflammatory responses. Computer searching (GCG) also revealed the presence of other potential sites, including the SLE.LP, the IRSE-like, the cAMP, and the GRE-like recognition elements. A total of 74 such potential sites were identified and as shown in Figure 2, 36 of these were in the 1500 bases immediately downstream of the *IL10.R* microsatellite.

Mapping of the human *IL10* gene to chromosome 1q

A previous comparison of the mouse and human *IL10* loci (Kim et al. 1992) localized the human *IL10* gene to chromosome 1. The mouse gene was localized more closely, lying in association with the REN, Ly-5 (CD45), and so on. Because of the recognized synteny between the mouse and human genomes in this area, it has been assumed that the human *IL10* gene lay in the same relative position as in the mouse, but this has never been determined. Our mapping results are summarized in Figure 3. The human *IL10* gene was positioned within 0.00cR of the WI-9614 framework marker 890.24cR from the tip of chromosome 1p. In the area close to WI-9641, a distance of 1.0 cR is approximately equal to 0.8 Mb (Whitehead Institute, personal communication). The *IL10.G* microsa-

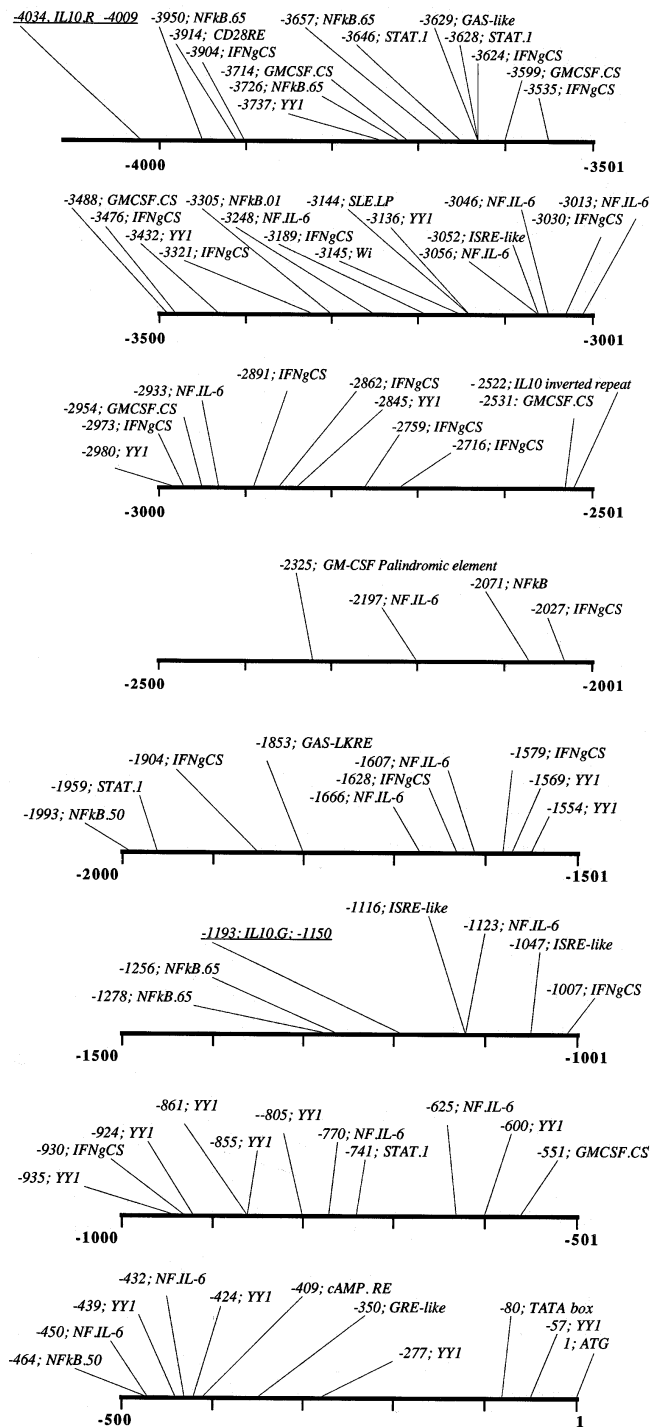


Fig. 2 The sites of potential transcription factor binding sites in the human *IL10* 5' flanking region are shown on this map. The position of the 5' end of the recognition sequence is shown. Note the concentration of these sites in the part of the gene close to the *IL10.R* microsatellite

tellite was positioned at 890.24cR and the *IL10.R* microsatellite at 891.24cR, with a LOD score of >3.0 in each case. Thus, some sense of directionality was implied and so we have indicated a possible transcriptional orientation (5'-3', towards the centromere), although this remains to be confirmed. The full vector analysis positioned the micro-

satellites in the same location as the following expressed sequence tag (EST) markers: WI-15562, NIB1751 and SGC35372 (examination of the sequences of these markers shows that none of them is *IL-10*). The nearest Genethon panel markers were D1S505 (5.23cR, telomeric) and D1S504 (5.17cR, centromeric). In cytogenetic terms, this probably places the human *IL10* gene at the junction of 1q31 and 1q32.

Definition of two new point mutations in the human IL10 promoter and their allelic distribution

As shown in Figure 4, Pileup analysis of the various sequences for the *IL10* promoter available from the GenBank/EMBL database suggested that three polymorphic areas might exist. Analysis of this observation (Materials and methods) revealed that two of these (identified by the enzymes *Mae* III and *Rsa* I) were, in fact, polymorphic. Amplification of the human *IL10* promoter with the IL-10.5 and IL-10.6 primers yielded a 486 base product. This fragment contains one conserved *Mae* III site in addition to the polymorphic one. Thus, digestion with *Mae* III yields 2 bands (361 and 125 bp) where nucleotide -854 is T and there is no additional cut site; where base -854 is C and there is an additional cut site, three bands are generated (217, 144, and 125 bp). Similarly, the amplified fragment contains three conserved *Rsa* I sites and so digestion with *Rsa* I yielded four bands (351, 85, 42, 8 bp) where base -627 is C and five bands (240, 111, 85, 42, 8 bp) where base -627 is A. We followed our convention of naming the cut allele "allele 1" and the uncut allele "allele 2" in each case. The widely available Tissue-Typing Panel cell-line IBW9 was homozygous for *IL10*.-854.1 and *IL10*.-627.2, while the cell-line LZL was homozygous for *IL10*.-854.2 and *IL10*.-627.1. The three possible combinations for each of these (homozygous cut, heterozygous, and homozygous uncut) were observed in our test population of 75 unrelated individuals and are shown in Figure 5. Also shown in Figure 5 is the fact that we failed to observe any polymorphism at base -686 (suggested by the Pileup analysis to be polymorphic for cutting with *Hpa* II); this may be a very rare allele. In our population, these new alleles were distributed thus: *IL10*.-854.1, 76.7%; *IL10*.-854.2, 23.3%; *IL10*.-627.1, 23.3%; *IL10*.-627.2, 76.7%. These alleles appeared to be in complete linkage equilibrium; we observed that *IL10*.-854.1 was always present with allele *IL10*.-627.2 and vice versa. When we examined the relationship between these new alleles and our previously characterized microsatellite alleles, we observed that the rare combination (*IL10*.-854.2 and *IL10*.-627.1) was strongly associated with the *IL10.G9* allele ($\chi^2 = 9.111$, $P = 0.0025$, $PC = 0.025$), while its association with the *IL10.R2* allele was slightly less strong ($\chi^2 = 5.359$, $P = 0.0206$, $PC = 0.0618$); no associations were observed with other common *IL10.G* alleles.

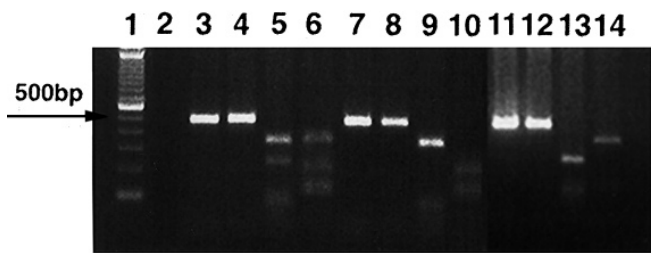


Fig. 5 Typical gels demonstrating the results from the *IL10.5/IL10.6* amplification and restriction enzyme digestion analysis. Track 1 shows the 100 bp ladder, with the 500 bp band indicated. Track 2 is empty. Results from donors A, B, and C are shown in tracks 3–6, 7–10, and 11–14, respectively. Tracks 3, 7, and 11 show undigested amplification product, tracks 4, 8, and 12 have been subjected to digestion with *Hpa* II, tracks 5, 9, and 13 with *Rsa* I, and tracks 6, 10, and 14 with *Mae* III. No digestion was observed with *Hpa* II for any DNA sample tested. Tracks 5, 9, and 13 represent individuals who were typed as *IL10.-627.1/2*; *IL10.-627.2/2*, and *IL10.-627.1/1*, respectively. Similarly, tracks 6, 10, and 14 represent the complimentary typing of *IL10.-854.1/2*; *IL10.-854.1/1* and *IL10.-854.2/2* in the same individuals

It was of interest that the NF κ B site located at -482 is in an area which we have recently shown to be a silencing region in EBV-positive Burkitt's lymphoma cells (Kube et al. 1995). Our recent data suggest that this silencing activity is placed in an area which also contains strong gene-activating signals (Kube and co-workers, unpublished observations). Recently, it has been shown that three of these NF κ B sites are able to bind NF κ B in HTLV *tax*-expressing human T-cells, suggesting a direct involvement of this transcription factor in virus-associated IL-10 expression (Mori et al. 1996).

We also noted two point mutations in the *IL10* promoter, at -854 and -627 respectively. In our population, these were in complete linkage equilibrium, as might be expected from their very close proximity to one another. When we examined their relationship with the *IL10.G* or *IL10.R* microsatellite alleles, a strong association was observed between the *IL10.G9* microsatellite allele and the less common *IL10.-854.2/IL10.-627.1* combination.

IL-10 is a central component of the immune response. It offers control over inflammatory (Malefyt et al. 1991b) and cell-mediated (Malefyt et al. 1991a) immunological mechanisms. This is reflected in its apparent involvement in many immunological disease states, especially malignant, autoimmune, and infectious diseases. Recent evidence has also shown that IL-10 is a target for many viruses in their attempts to subvert the human immune system. The area of chromosome 1 where human IL-10 is encoded is rich with genes encoding proteins relevant to the immune system; for example, components of the complement system (decay accelerating factor, complement receptor 1, complement receptor 2) are adjacent to our location for the *IL10* gene as are those for cell-surface structures such as CD21. The genes for many of the Fc-gamma receptors are also nearby (1q23). As reports begin to emerge about the role of elements within this part of chromosome 1 in the genetic background to malignant and autoimmune diseases (Bieche et al. 1995; Salmon et al. 1995; Eskdale et al. 1997), so the accurate positioning of the *IL10* gene in relation to such

others will assist in the understanding of its role (if any) in the molecular aetiology of disease, and its relationship with other parts of the immune system generally, as will a full understanding of the genetic diversity which exists within this important gene. Like other cytokines, IL-10 does not act in isolation but functions within a cytokine network. The uncovering of a dense clustering of sites through which other pro-inflammatory cytokines might influence the production of IL-10 provides a potential mechanism for this.

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Note added in proof:

After this paper had been accepted for publication, we became aware of independent work by Turner and co-workers (1997) in which they note the two point mutations described here. We acknowledge the contribution by Turner and co-workers to the understanding of the structure of the human *IL10* promoter.