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A second polymorphic dinucleotide repeat in the 5' flanking region of the human *IL10* gene

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Interleukin-10 (IL10) is an important multifunctional cytokine, secreted by many cell types and to which a number of cell types are responsive (Moore 1992). IL-10 is steadily assuming a role as a central cytokine in the control of macrophage function and inflammatory responses, through its ability to regulate the production of tumour necrosis factor (TNF) and other pro-inflammatory cytokines (de Waal Malefyt et al. 1991). Following induction in macrophages, the TNF subsequently induces IL-10 in the same cells, repressing TNF transcription in an autocrine loop (Platzer et al. 1995). In addition to these inhibitory effects on TNF production, IL-10 may also promote inflammatory responses through effects on B cells. Raised IL-10 levels have been reported in several autoimmune conditions (Llorente et al. 1994). IL-10 is a potent activator of B cells, stimulating both proliferation and differentiation (Rousset et al. 1992). 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).

The effects of IL-10 on B-cell function also appear to be important in the aetiology of human lymphoma, where its expression can be profoundly deregulated. Infection and transformation of human B cells by Epstein-Barr virus (EBV) induces the production of human 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). Furthermore, IL-10 functions as an autocrine growth factor in HIV-related B-cell lymphoma (Masood et al. 1995).

The potential importance of IL-10 in the epidemiology of autoimmune diseases and lymphoid malignancies has led us to search for polymorphic elements within the *IL10* locus, which may

provide insights into this cytokine's involvement in genetic predisposition in human disease. We have recently described a highly polymorphic and informative microsatellite marker (*IL10.G*) in the *IL10* promoter (Eskdale and Gallagher 1995). In the present report, we describe a second polymorphic dinucleotide repeat (*IL10.R*) in the 5' flanking region of the *IL10* gene and discuss its relationship with the first.

The *IL10.R* microsatellite lies between –4004 and –3978 of the human *IL10* 5' flanking region sequence, HSINTL10 (numbering relative to the translation initiation site in GenEMBL X78437, 1996 update; D. Kube), originally described by Kube and co-workers (1995). It is approximately 2800 base pairs (bp) upstream of the *IL10.G* microsatellite (–1193 to –1151). We examined this CA repeat for evidence of polymorphism in 94 unrelated individuals, drawn from the same group used for our previous characterization of the *IL10.G* microsatellite. Genomic DNA was amplified using a primer extension technique and the following oligonucleotide primers (Cruachem, Glasgow, Scotland):

IL-10.3, upstream; 5' CCC.TCC.AAA.ATC.TAT.TTG.CAT.AAG 3'
IL-10.4, downstream; 5' CTC.CGC.CCA.GTA.AGT.TTC.ATC.AC 3'

The upstream primer (IL-10.3) lies 1 bp from the start of the repeat sequence and the downstream primer (IL-10.4) begins 41 bp from the end of the CA repeat.

In addition to the test DNA, each reaction contained: 1 µM each primer; 200 µM each dATP, dGTP, dTTP, and 20 µM dCTP (Pharmacia, Milton Keynes, England); 0.5 units Primezyme (Biometra, Göttingen, Germany), in 1 × reaction buffer with 1.5 mM MgCl₂ (Biometra); α³²P-dCTP (Amersham International, Amersham, UK) was added to label the reaction product internally. The final reaction volume was 20 µl. After an initial melting time of 5 min, samples were subjected to 30 rounds of 94 °C, 15 s; 61 °C, 15 s; 72 °C, 15 s in a Biometra Uno Thermoblock. Samples were mixed with formamide loading buffer, heated to 80 °C for 10 min, then cooled immediately on ice, prior to separation on a sequencing gel containing 6% acrylamide (19:1; Gibco, Paisley, Scotland) and 7 M urea (Appligene, Strasbourg, France) at 75W on a Stratagene Baseace apparatus (Stratagene), usually for 3 h. These conditions have been optimized for our reagents and equipment and we would recommend that others do the same. After drying, gels were allowed to expose X-ray film and the resultant alleles were called after direct sizing against an end-labelled 10 bp ladder (Gibco), run in two or three lanes of the gel.

The polymorphism data reported in this paper have been submitted to the human genome database and have been assigned the accession number GDB: 1386733

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Table 1 Relationship between certain *IL10R* and *IL10G* alleles. Allele association in 92 unrelated individuals was scored in a series of 2×2 tables which were then analysed for association by the chi-square test. The significance level (*P* value) was then multiplied by three to correct for the number of observed *IL10.R* alleles (pc-value). Relationships where *PC* < 0.05 were considered significant

<i>IL10.R</i> allele	<i>IL10.G</i> allele	Chi-square value	<i>P</i> value	<i>PC</i> value
<i>IL10.R2</i>	<i>IL10.G11</i>	8.354	<i>P</i> = 0.0038	<i>PC</i> = 0.0114
<i>IL10.R2</i>	<i>IL10.G13</i>	17.454	<i>P</i> < 0.00001	<i>PC</i> < 0.00003
<i>IL10.R3</i>	<i>IL10.G9</i>	12.582	<i>P</i> = 0.0004	<i>PC</i> = 0.0012
<i>IL10.R3</i>	<i>IL10.G10</i>	8.119	<i>P</i> = 0.0044	<i>PC</i> = 0.0132

We identified three alleles at this locus in our population, whose sizes are 114 bp, 116 bp, and 118 bp respectively, relative to the 10 bp ladder. We originally termed these "1, 2, 3"; however, studies of a different ethnic population revealed the presence of two additional alleles, both very rare. One is smaller than our original "allele 1" and one is larger than our original "allele 3" (these data will be reported elsewhere). We therefore termed the alleles reported in this study *IL10.R2*, *IL10.R3*, and *IL10.R4* to acknowledge this fact. As before (Eskdale and Gallagher 1995), we have assumed no null alleles. Allele *IL10.R4* (3.2%) is itself rare in our population, while *IL10.R2* (56.4%) and *IL10.R3* (40.4%) are both common. The total heterozygosity observed was 50% and these were mostly *IL10.R2/3* heterozygotes (43.6% of the total test population). The widely available tissue-typing panel cell-lines OLL and LZL are both homozygous for allele *IL10.R2* (not shown).

We examined the relationship of alleles at the *IL10.G* locus with those at the *IL10.R* locus in 92 unrelated individuals by constructing a series of 2×2 tables and testing the apparent associations by chi-square analysis using Minitab software on an Apple Macintosh computer. As shown in Table 1, four significant associations were observed: *IL10.R2 + IL10.G11*;
IL10.R2 + IL10.G13;
IL10.R3 + IL10.G9;
IL10.R3 + IL10.G10.

While not definitive, these data suggest that the alleles involved in these associations may usually be carried on the same strand of DNA and constitute haplotypes. Of the 92 individuals typed at both *IL10.R* and *IL10.G*, 30 carried two of these putative haplotypes and a further 52 carried one of them, thereby supporting this hypothesis.

A recent report suggests that individual variations in the levels of secreted IL-10 may have pathological significance (Huizinga et al. 1996); evidence from the TNF system supports the concept that heritable differences in cytokine secretion exist and demonstrates that dinucleotide repeat polymorphisms can be useful markers of such events (Pociot et al. 1994). Whether this is the case for the *IL10* locus remains to be seen but we believe that the polymorphic CA repeats we describe here (and described previously, see Eskdale and Gallagher 1995) will be useful tools in determining the role (if any) of IL-10 in genetic susceptibility to leukaemic and inflammatory disorders. Its apparent ability to protect B cells from apoptosis (Levy et al. 1994) may be relevant in both these scenarios. The highly polymorphic nature of the *IL10* promoter may therefore allow new understanding across a range of pathological conditions which involve human B cells and the dysregulation of their normal function.

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