Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease

J Grove, A K Daly, M F Bassendine, E Gilvarry, C P Day

Abstract

***Background*—The factors determining why less than 10% of heavy drinkers develop advanced alcoholic liver disease (ALD) remain elusive, although genetic factors may be important. Interleukin 10 (IL-10) is an important cytokine with anti-inflammatory,anti-immune,andanti-fibrotic functions. Several polymorphisms have been identified in the IL-10 promoter and recent evidence suggests that some of these may have functional effects on IL-10 secretion.**

***Aims*—To test the hypothesis that IL-10 promoter region polymorphisms are associated with susceptibility to ALD.**

***Methods*—The allele frequencies for the two single base pair substitutions at positions −627 (C→A) and −1117 (A→G) in the IL-10 promoter were determined in 287 heavy drinkers with biopsy proved advanced ALD, 107 heavy drinkers with no evidence of liver disease or steatosis only on biopsy, and 227 local healthy volunteers.**

***Results*—At position −627, 50% of patients with advanced ALD had a least one A allele compared with 33% of controls ($p<0.0001$) and 34% of drinkers with no or mild disease ($p=0.017$). At position −1117, the slight excess of the A allele in drinkers with advanced disease was because of linkage disequilibrium between the A alleles at the two sites.**

***Conclusions*—Among heavy drinkers, possession of the A allele at position −627 in the IL-10 promoter is associated with an increased risk of advanced liver disease. This is consistent with recent functional data that the −627*A allele is associated with low IL-10 expression which will favour inflammatory, immune mediated, and profibrotic mechanisms of alcohol related liver injury.**

(*Gut* 2000;**46**:540–545)

Keywords: ethyl alcohol; cirrhosis; interleukin 10; genetic polymorphism

Centre for Liver Research, University of Newcastle, Newcastle-upon-Tyne, UK
J Grove
M F Bassendine
C P Day

Department of Pharmacological Sciences
A K Daly

Department of Psychiatry
E Gilvarry

Correspondence to:
Dr C P Day, Department of Medicine, The Medical School, Framlington Place, Newcastle-upon-Tyne NE2 4HH, UK

Accepted for publication 22 October 1999

Alcoholic liver disease (ALD) progressing to fibrosis and cirrhosis develops in less than 10% of heavy drinkers.[1] Although some evidence supports a dose-response relation between alcohol consumption and risk of disease,[1 2] it is clear that other factors determine which drinkers progress beyond the stage of simple steatosis; a study of almost 16 000 male twin pairs has suggested that disease susceptibility may have a genetic component.[3 4] Hitherto, studies looking for "susceptibility genes" have largely focused on genes encoding ethanol metabolising enzymes.[5 6] Results have been conflicting, but it would seem that the known polymorphisms of genes encoding the alcohol and aldehyde dehydrogenase enzymes and the ethanol inducible cytochrome P450, CYP2E1, play only a minor role in Caucasoid populations.[7–9] More recently increasing evidence supporting a role for cytokines in the pathogenesis of ALD[10] has suggested an alternative set of "candidate genes" with a potential role in disease susceptibility. Considering its undoubted significance in the pathogenesis of ALD, not surprisingly the recently described promoter region polymorphisms in tumour necrosis factor α (TNFα) have been the first cytokine polymorphisms to be studied for their association with ALD.[11–13] The only large published study has reported an association between the −238 G→A polymorphism (present in 7% of controls) and alcoholic steatohepatitis,[13] the significance of which awaits more information on the functional significance of the polymorphism.

The cytokine interleukin 10 (IL-10) has recently emerged as an important suppressor factor for both immunoproliferative and inflammatory responses.[14] It is produced by numerous cell types including activated Th0, Th1, and Th2 CD4+ T helper cells, cytotoxic CD8+ T cells, monocytes, Kupffer cells,[15] hepatocytes,[16] and hepatic stellate cells.[17] IL-10 inhibits antigen specific activation, proliferation, and cytokine production by Th0, Th1 (IL-2 and interferon γ (IFNγ)), and Th2 (IL-4, IL-5) clones by reducing the antigen presenting capacity of monocytes, associated with down regulation of class II HLA molecules and B7 expression on their surface.[18–20] In contrast, in B lymphocytes, IL-10 stimulates proliferation, immunoglobulin secretion, and isotype class switching from IgM to IgA.[21 22] IL-10 also exerts potent anti-inflammatory effects. It down regulates the synthesis of proinflammatory cytokines and chemokines by monocytes and Kupffer cells stimulated by endotoxin, including IL-1, TNFα, IL-6, IL-8, and IL-12, and up regulates the synthesis of the IL-1R antagonist.[15 23 24] Neutrophil chemotaxis and chemokine expression are also down regulated.[25] Finally, IL-10 may also exert antifibrotic effects in the liver through inhibition of

Abbreviations used in this paper: ALD, alcoholic liver disease; IL-10, interleukin 10.

collagen gene transcription and increased collagenase expression by hepatic stellate cells.[26] In vivo evidence for the anti-inflammatory and antifibrotic properties of endogenous IL-10 has recently been provided by animal models of liver injury,[27–30] including studies in IL-10 gene knockout mice that have shown increased fibrosis[15 31] and neutrophil infiltration[31] following chronic carbon tetrachloride administration. As immune mediated mechanisms,[32] the release of proinflammatory cytokines from endotoxin stimulated Kupffer cells,[10] and increased fibrogenesis have all been implicated in the pathogenesis of ALD, it seems highly probable that IL-10 plays a significant role in influencing the development of liver injury following excessive alcohol consumption.

IL-10 secretion following lipopolysaccharide stimulation of whole blood in vitro seems to vary widely between individuals, and twin studies have suggested that more than 70% of this variation is genetically determined at the transcriptional level.[33] The IL-10 gene is located on the long arm of chromosome 1 in the 1q32 band and has a highly polymorphic 5' flanking region. Thus far there have been three single base pair substitutions identified (1117, 854, and 627 bases upstream of the transcriptional start site), and two microsatellite loci (IL10.G and IL10.R, located 1.2 kb and 4.0 kb upstream respectively).[34 35] In "healthy" individuals nucleotides A and G are present at approximately similar frequencies at position −1117, while a C→A substitution at position −627 is present in 21−23% and is in complete linkage disequilibrium with a C→T substitution at position −854.[34 35] Linkage also exists between the −627*A allele and particular IL10.R and IL10.G alleles.[35] In Caucasians, the three base pair substitutions have so far been reported to produce only three different haplotypes: GCC, ACC, and ATA.[34]

Indirect evidence for an effect of these polymorphisms on IL-10 gene expression has come from disease association studies. The ATA haplotype and −627*A allele are associated with severe asthma,[36 37] which is characterised by low concentrations of IL-10,[38] while the GCC haplotype and −627*C allele are associated with renal disease in sytemic lupus erythematosus,[39] characterised by high concentrations of IL-10. Most recently the ATA haplotype and the −627*A allele has been associated with response to interferon in patients with chronic hepatitis C.[40] This is also consistent with these patients having lower concentrations of IL-10, resulting in a more vigorous immune response to hepatitis C virus (HCV). More direct evidence has come from studies correlating IL-10 genotype with IL-10 secretion by peripheral blood mononuclear cells or whole blood. Consistent with the disease association studies, higher IL-10 expression has been associated with the −627*C allele[41] and the GCC haplotype,[34 40 42] and this has recently been confirmed in transient transfection studies comparing the ATA and GCC haplotypes.[42] In view of the immunomodulatory, anti-inflammatory, and antifibrogenic effects of IL-10, we hypothesised that possession of IL-10 alleles associated with low IL-10 production would increase susceptibility to alcoholic liver disease. We sought to test this hypothesis by comparing the allele frequencies for the base pair substitutions at positions −627 and −1117 in patients with histologically proved fibrotic ALD with those in locally matched healthy controls and alcoholics with either no liver disease or fatty liver (steatosis) only.

Methods

PATIENTS

After obtaining informed consent, blood (10 ml) was collected from heavy drinkers and healthy controls. All drinkers and controls were Caucasoid and originated in the north-east of England as did their parents and grandparents. Heavy drinkers were recruited from two sources: referrals to the hospital liver unit with suspected ALD; and referrals to the Regional Alcohol Addiction Unit with alcohol dependency. Patients who presented to the liver unit had their detailed lifetime alcohol history taken by a specific alcohol research nurse, and those who presented to the Alcohol Addiction Unit by a community psychiatric nurse trained in drug and alcohol addiction. To be included in the study patients had to have been consuming more than 80 g ethanol/day for at least 10 years at the time of presentation. The presence and severity of ALD was determined initially by standard liver blood tests. Patients with either alanine transaminase, alkaline phosphatase, or bilirubin more than twice the upper limit of normal on two occasions within a six month period were further investigated with ultrasonography. If this showed no evidence of biliary obstruction, liver biopsy was performed unless contraindicated by coagulopathy (prothrombin time more than three seconds prolonged).

On the basis of these investigations, drinkers were placed into two categories: those with advanced ALD (AALD group), and those with no evidence of alcoholic liver disease or simple steatosis only (NALD group). The criteria for inclusion into the AALD group were either liver histology compatible with ALD of greater severity than simple steatosis (see below), or clinical evidence of hepatic decompensation. For inclusion into the NALD group patients had to be actively drinking, have no clinical evidence of liver disease, and have either: normal liver blood tests on two occasions (not including an isolated rise in γ-glutamyl transferase)[43]; or, for those with abnormal liver blood tests, liver histology showing either normal liver or steatosis with no evidence of steatohepatitis or fibrosis.

Drinkers were excluded from this study if they had any of the following: serological evidence of previous HBV or HCV infection (second generation ELISA) or autoimmune liver disease; histological evidence of other liver diseases; excessive liver iron staining and homozygosity for the C282Y mutation in the *HFE* gene[44]; or "mild" abnormalities of liver blood tests (bilirubin, alanine transaminase,

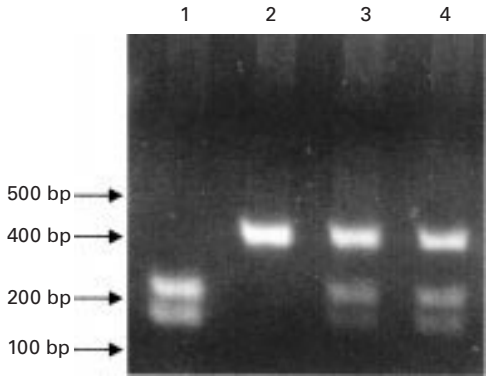

Figure 1 Genotyping for the C-627A polymorphism by PCR-RFLP (restriction fragment length polymorphism) analysis. Samples were amplified by PCR followed by digestion with Rsa I. Lane 1, homozygous mutant (AA); lane 2, homozygous wild type (CC); lane 3, heterozygous (CA); lane 4, heterozygous (CA).

alkaline phosphatase less than twice normal). It was not considered ethically justifiable to biopsy these individuals and impossible to classify them as AALD or NALD without a biopsy. Healthy control subjects were recruited from hospital and university staff. None was drinking more than "sensible" limits (21 units per week for men and 15 units per week for women).

HISTOLOGY

Biopsy specimens were categorised according to the presence or absence of: cirrhosis; precirrhotic perivenular/pericellular fibrosis; and steatohepatitis, defined as the presence of steatosis, ballooning degeneration, and Mallory's hyaline with or without neutrophil satellitosis. In the absence of liver histology only patients with definite features of decompensation (varices, ascites, or encephalopathy) were categorised as cirrhotic. No attempt was made to diagnose precirrhotic fibrosis or steatohepatitis without liver biopsy.

DETERMINATION OF IL-10 GENOTYPE

For the −627 polymorphism, polymerase chain reaction (PCR) was used to amplify a 412 bp fragment containing the −627 IL-10 promoter polymorphism in 50 µl of KCl buffer (Bioline, London, UK) containing 1 µg test DNA, 200 µM dNTPs, 0.25 µM primer 627A (5'-CCTAGGTCACAGTGACGTGG), 0.25 µM primer 627S (5'-GGTGAGCACTACCT GACTAGC), and 2 units *Taq* polymerase (Bioline). The PCR conditions consisted of 35 cycles at 94°C for one minute, 50°C for one minute, and 70°C for one minute, followed by one cycle at 70°C for one minute. A 10 µl aliquot of the resulting product was incubated at 37°C for 16 hours with *Rsa*I (2 units), which only cleaves DNA fragments with an A at position −627, and electrophoresed on a 2% agarose gel. Each batch of reactions included a control reaction to which no DNA had been added, to ensure that no contamination of samples had occurred, and two control samples of known genotype (for example, see fig 1).

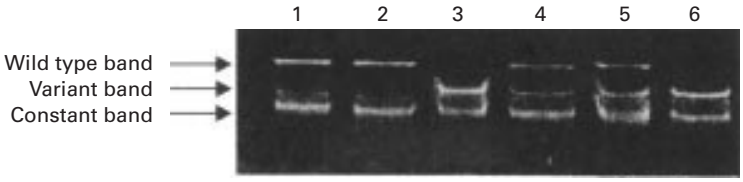

Figure 2 Genotyping for A-1117G polymorphism by SSCP analysis. Samples were amplified by PCR followed by denaturation and analysis by SSCP on an MDE gel. A constant band seen with both genotypes is indicated together with two bands which represent the wild type allele (A) and variant allele (G) respectively. Lane 1, homozygous wild type (AA); lane 2, homozygous wild type (AA); lane 3, homozygous mutant (GG); lane 4, heterozygous (AG); lane 5, heterozygous (AG); lane 6, homozygous mutant (GG).

The −1117 promoter polymorphism was detected following amplification of a 321 bp DNA fragment by PCR using primers 1117A (5'-AAGCTTCTGTGGCTGGAGTC) and 1117S (5'-CCAGAGACTTTCCAGATATC TGAAGAAG) using the same PCR conditions as for the −627 polymorphism. The −1117 genotype was determined using single strand conformational polymorphism (SSCP) analysis of the PCR products on a 1× MDE gel (200 mm × 200 mm × 1.5 mm) in accordance with the manufacturer's recommendations (FMC BioProducts, Massachusetts). Samples were electrophoresed at 200 V and 4°C for 16 hours, and stained with ethidium bromide. PCR products from individuals with GG, GA, and AA genotypes were identified by direct sequencing of the PCR products using a sequenase PCR product sequencing kit (USB, Ohio), and were included on each SSCP gel acting as "standards", allowing genotypes to be assigned to unknown samples on the gels (for example, see fig 2).

STATISTICS

Differences between genotypes and allele frequencies and deviations from the Hardy-Weinberg equilibrium were analysed using the χ^2 test or Fisher's exact test, depending on the minimum expected values. Odds ratios (OR) with 95% confidence intervals (CI) were also calculated where appropriate. As we were examining two polymorphisms, $p<0.025$ was considered statistically significant.

Results

PATIENT GROUPS

Since August 1990, 287 heavy drinkers have been recruited into the AALD group (206 men). In 230 cases this classification was made following a diagnostic liver biopsy (n=223) or postmortem examination (n=7); a further 57 cases were classified as cirrhotic on the basis of clinical evidence of severe portal hypertension (oesophageal varices or ascites). In the 230 patients with liver histology available, 177 had established cirrhosis and 53 precirrhotic fibrosis. Coexisting alcoholic steatohepatitis was present in 80 of the cirrhotic patients and 30 of those with precirrhotic fibrosis. A total of 107 heavy drinkers have been recruited into the NALD group (76 men). Compared with the AALD group, the difference in age was significant ($p<0.001$), while the sex distribution was identical. In 18 cases the classification as NALD was made following a diagnostic liver biopsy showing fatty liver only. The remaining 89 cases had two sets of normal liver blood tests while actively drinking. The majority

Table 1 Distribution of the −627 interkeukin (IL) 10 polymorphism in healthy controls and heavy drinkers with and without advanced liver disease

Subjects	*AA*	*AC*	*CC*
Controls (n=227)	5 (2.2%)	70 (30.8%)	152 (67.0%)
AALD (n=287)	19 (6.6%)	125 (43.6%)	143 (49.8%)*
NALD (n=107)	3 (2.8%)	36 (33.6%)	68 (63.6%)

*Odds ratio (95% confidence interval) for possession of one or two A alleles versus controls: 2.04 (1.42 to 2.92); versus NALD: 1.76 (1.12 to 2.77).
AALD, advanced alcoholic liver disease; NALD, no evidence of alcoholic liver disease or steatosis only.

Table 2 Distribution of the −1117 interleukin (IL) 10 polymorphism in healthy controls and heavy drinkers with and without advanced liver disease

Subjects	*AA*	*AG*	*GG*
Controls (n=212)	44 (20.8%)	102 (48.1%)	66 (31.1%)
AALD (n=285)	72 (25.3%)	149 (52.3%)	64 (22.5%)
NALD (n=107)	23 (21.5%)	53 (49.5%)	31 (29.0%)

AALD, advanced alcoholic liver disease; NALD, no evidence of alcoholic liver disease or steatosis only.

(n=74) of these drinkers were recruited from the alcohol addiction unit.

IL-10 GENOTYPE

Table 1 shows the frequency distribution of the −627 IL-10 polymorphism genotypes in both patient groups and healthy controls. The distribution of genotypes in patients and controls was in Hardy-Weinberg equilibrium. A significant excess of AA homozygotes and AC heterozygotes was observed in AALD patients compared with both controls and NALD patients (AA/AC: 50% AALD versus 33% controls, p<0.0001, OR (95% CI) 2.04 (1.42 to 2.92); versus 36% NALD, p=0.017, OR (95% CI) 1.76 (1.12 to 2.77)). These genotypes gave corresponding A allele frequencies of 0.284 in AALD patients, 0.176 in controls, and 0.196 in NALD patients (AALD versus controls, p<0.0001; versus NALD, p=0.013). The genotype frequency in AALD patients did not differ according to either sex or the presence of steatohepatitis.

Table 2 shows the frequency distribution of the −1117 IL-10 polymorphism genotypes in both patient groups and healthy controls. The distribution of genotypes in patients and controls was in Hardy-Weinberg equilibrium. Drinkers with AALD had a slight increase in the frequency of AA/AG genotypes compared with controls (77.5% versus 69%, p=0.03, OR (95% CI) 1.56 (1.04 to 2.3)) but not with NALD drinkers (71%, p=0.19). This observation is explained by the tight linkage disequilibrium that exists between the A alleles at the two positions reported previously[23] and confirmed in this study. Of the 310 assignable haplotypes in the controls, 30/31 A alleles at position −627 were associated with an A at position −1117 with only 14/31 predicted from the −1117 A allele frequency. In contrast to previous reports in whites,[34] we observed at least eight alleles (one control, one NALD, six AALD) with the A-627/G-1117 haplotype and therefore did not consider it feasible to assign haplotypes to alleles from individuals heterozygous at both positions.

Discussion

In this case control study we have shown a strong association between possession of the A allele at position −627 in the IL-10 promoter region and fibrotic ALD. To our knowledge this is the first liver disease association reported for any of the recently described IL-10 upstream promoter region polymorphisms. A weak association was also observed with the A allele at position −1117 but this was owing to the tight, although incomplete, linkage disequilibrium that exists between the A alleles at the two polymorphic sites. Recent direct and indirect evidence suggests that the −627*A allele/ATA haplotype is associated with low IL-10 expression,[34 36–42] confirming our hypothesis that drinkers with genetically determined low IL-10 secretion would be at increased risk of developing ALD.

A case control allelic association study such as this is subject to a number of methodological problems both peculiar to ALD and to this type of study in general. Perhaps the biggest problem in studies of ALD is the selection of "alcoholic controls" who have not developed advanced disease despite excessive alcohol intake. Determining the absence of significant liver disease is imprecise without a liver biopsy which may be unethical in patients with normal liver blood tests. However, a previous biopsy study in asymptomatic alcoholics showed that only four of 85 with normal blood tests and no clinical evidence of liver disease had either alcoholic hepatitis or cirrhosis.[45] It seems reasonable to assume, therefore, that the proportion of our NALD group not undergoing liver biopsy and yet having advanced liver disease is likely to be very small and will not have significantly affected the results. A further problem in selecting the NALD group is posed by the relation between cumulative lifetime alcohol dose and ALD risk.[1 2] This implies that the alcohol intake of the NALD group should be similar to that of drinkers with advanced liver disease. In this study the selection of drinkers with at least a 10 year history of 80 g/day intake at the time of presentation suggests that the level of intake was similar in patients with and without advanced liver disease.

There are at least three potential pitfalls common to all studies using the current methodology. The first is that of population stratification or the "founder effect". This implies that the observed association is caused by our cases and controls coming from different founder (ethnic) populations with different susceptibilities to ALD and different frequencies of the IL-10 −627*A allele en passant. The two ways to tackle this problem are to repeat the observation in an ethnically distinct population and/or to look for intrafamilial allelic association using the transmission disequilibrium test (TDT).[46] Unfortunately familial studies are difficult to perform in ALD, a stigmatised disease largely presenting in middle age. However, against population stratification as an explanation for our results is both the careful local matching of cases and controls in an area of little population movement, and our

previous results showing identical frequencies of polymorphisms in the HFE,[44] type I collagen,[47] and α_1 antitrypsin[48] gene loci in similarly selected cases and controls.

The second pitfall is that of chance associations occurring owing to multiple allele testing as has been seen in HLA association studies.[49] However, the primary aim of this study was to look for an association between either of the two IL-10 upstream region polymorphisms and ALD and the p values remained significant after correcting for two allele testing ($p<0.025$).

The third and final pitfall is that the observed association with disease is indirect and reflects linkage disequilibrium with another polymorphism in either the same or a neighbouring gene. Clearly, the tightly linked IL-10 polymorphism at position −854 is a good "candidate" in this respect,[34] along with a number of other candidate genes that map to chromosome 1q32, including: TNF receptor associated factor 5 (TRAF5), complement binding proteins, and laminin, an essential component of the extracellular matrix. The two microsatellite polymorphisms also seem worthy of study as they have recently been associated with differential IL-10 secretion[50] and susceptibility to rheumatoid arthritis.[51] The emerging functional data on the effect of the single base pair substitutions on IL-10 gene transcription[42] and the increased understanding of the role of IL-10 in liver injury does, however, suggest that the disease association with the low IL-10 producing −627*A allele is direct. Our allelic association data provide further evidence that the reported association between the ATA haplotype and low IL-10 secretion[40 42] is an association with the −627*A allele[41] rather than the −1117*A allele.[34]

Defining the particular mechanisms by which low IL-10 concentrations contribute to the development of ALD is difficult in the absence of any clear consensus on disease pathogenesis. One attraction of IL-10 in determining susceptibility to ALD, however, is that it potentially affects most if not all of the mechanisms currently considered to be important. For example, low concentrations of IL-10 would be expected to increase: the release of proinflammatory cytokines by endotoxin stimulated Kupffer cells[23 24]; neutrophil chemotaxis and chemokine secretion[25]; superoxide production by Kupffer cells[52]; the antigen presentation of alcohol related neoantigens[32] by monocytes to, and the subsequent activation of, T cells[18–20]; and collagen gene transcription by activated hepatic stellate cells.[26] Clearly, these mechanisms play a role in the pathogenesis of many other liver diseases and therefore determining which of these diseases are also associated with the −627*A allele in the IL-10 promoter region may provide valuable insight into the pathogenesis of ALD. For the present, this polymorphism has the potential to be the first of several susceptibility loci for ALD capable of predicting disease risk and allowing appropriate advice to be given to individuals on alcohol intake prior to the onset of excessive consumption.

This work was supported by the UK Medical Research Council. We thank Margaret Hewitt RGN for her assistance in collecting and logging the samples.

1 Bellantani S, Costa G, Saccoccio G, *et al.* Drinking habits as cofactors of risk for alcohol-induced liver damage. *Gut* 1997;**41**:845–50.
2 Becker U, Deis A, Sorensen TIA, *et al.* Prediction of risk of liver disease by alcohol intake, sex and age: a prospective population study. *Hepatology* 1996;**23**:1025–9.
3 Hrubec Z, Omenn GS. Evidence of a genetic predisposition to alcoholic cirrhosis and psychosis; twin concordances for alcoholism and its biological end points by zygosity among male veterans. *Alcohol Clin Exp Res* 1984;**9**:306–9.
4 Reed T, Page WF, Viken RJ, *et al.* Genetic predisposition to organ specific endpoints of alcoholism. *Alcohol Clin Exp Res* 1996;**20**:1528–33.
5 Whitfield JB. Meta-analysis of the effects of alcohol dehydrogenase genotype on alcohol dependence and alcoholic liver disease. *Alcohol Alcohol* 1997;**32**:613–19.
6 Bassendine MF, Day CP. The inheritance of alcoholic liver disease. *Baillieres Clin Gastroenterol* 1998;**12**:317–35.
7 Day CP, James OFW, Bassendine MF, *et al.* Alcohol dehydrogenase polymorphisms and predisposition to alcoholic cirrhosis. *Hepatology* 1993;**18**:230–1.
8 Grove J, Daly AK, Brown AStJM, *et al.* The *Rsa* I polymorphism of CYP2E1 and susceptibility to alcoholic liver disease in Caucasians: effect on age of presentation and dependence on alcohol dehydrogenase genotype. *Pharmacogenetics* 1998;**8**:335–42.
9 Carr LG, Hartleroad JY, Liang YB, *et al.* Polymorphism at the P450IIE1 locus is not associated with alcoholic liver disease in Caucasian men. *Alcohol Clin Exp Res* 1995;**19**: 182–4.
10 Jarvelainen HA, Fang C, Indelman-Sundberg M, *et al.* Effect of chronic coadministration of endotoxin and ethanol on rat liver pathology and proinflammatory and anti-inflammatory cytokines. *Hepatology* 1999;**29**:1503–10.
11 Grove J, Daly AK, Bassendine MF, *et al.* Association of a tumor necrosis factor polymorphism with susceptibility to alcoholic steatohepatitis. *Hepatology* 1997;**26**:143–6.
12 Ramsay LA, Smith S, Taylor C, *et al.* Susceptibility to alcoholic liver disease (ALD) and tumour necrosis factor (TNF-alpha) promoter polymorphisms [abstract]. *J Hepatol* 1996;**25**(Suppl.1):110.
13 Allott RL, Quest LJ, Pirmohammed M, *et al.* Investigation of the role of the TNFα gene polymorphism in alcoholic liver disease (ALD) [abstract]. *Hepatology* 1996;**24**:443A.
14 Moore KW, O'Garra AO, de Waal Malefyt R, *et al.* Interleukin-10. *Ann Rev Immunol* 1993;**11**:165–90.
15 Thompson K, Maltby J, Fallowfield J, *et al.* Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis. *Hepatology* 1998;**28**:1597–606.
16 Alfrey EJ, Most D, Wang X, *et al.* Interferon-gamma and interleukin-10 messenger RNA are up-regulated after orthotopic liver transplantation in tolerant rats: evidence for cytokine-mediated immune dysregulation. *Surgery* 1995;**118**:399–405.
17 Thompson K, Trowern A, Fowell A, *et al.* Primary rat and mouse hepatic stellate cells express the macrophage inhibitor cytokine interleukin-10 during the course of activation in vitro. *Hepatology* 1998;**28**:1518–24.
18 Del Prete GM, DeCarli F, Almerigogna MG, *et al.* Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993;**150**:353–60.
19 de Waal Malefyt RJ, Haanen H, Spits MG, *et al.* Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen presenting capacity of monocytes via down regulation of class II major histocompatibility complex expression. *J Exp Med* 1991;**174**:915–24.
20 Willems F, Marchant A, Delville JP, *et al.* Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur J Immunol* 1994;**24**:1007–9.
21 Rousset FE, Garcia T, Defrance C, *et al.* Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci USA* 1992;**89**: 1890–3.
22 Defrance T, Vanbervliet B, Briere F, *et al.* Interleukin 10 and transforming growth factor beta cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J Exp Med* 1992;**175**:671–82.
23 de Waal Malefyt RJ, Abrams J, Bennett B, *et al.* Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991;**174**:1209–20.
24 Fiorentino DF, Zlotnik A, Mosmann TR, *et al.* IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991;**147**:3815–22.
25 Kasama T, Strieter RM, Lukacs MW, *et al.* Regulation of neutrophil derived chemokine expression by IL-10. *J Immunol* 1994;**152**:3559–69.
26 Wang SC, Ohata M, Scrum L, *et al.* Expression of interleukin-10 by in vitro and in vivo activated hepatic stellate cells. *J Biol Chem* 1998;**273**:302–8.
27 Arai T, Hiromatsu K, Kobayashi N, *et al.* IL-10 is involved in the protective effect of dibutyryl cyclic adenosine monophosphate on endotoxin-induced inflammatory liver injury. *J Immunol* 1995;**155**:5743–9.
28 Gazzinelli RT, Wysocka M, Hieny S, *et al.* In the absence of endogenous IL-10, mice acutely infected with Toxoplasma

gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-γ and TNF-α. *J Immunol* 1996;**157**:798–805.
29 Louis H, Le Moine O, Peny MO, *et al.* Production and role of interleukin-10 in concanavalin A-induced hepatitis in mice. *Hepatology* 1997;**25**:1382–9.
30 Louis H, Le Moine O, Peny MO, *et al.* Hepatoprotective role of interleukin 10 in galactosamine/lipopolysaccharide mouse liver injury. *Gastroenterology* 1997;**112**:935–42.
31 Louis H, Van Laethem JL, Wu W, *et al.* Interleukin-10 controls neutrophilic infiltration, hepatocyte proliferation, and liver fibrosis induced by carbon tetrachloride in mice. *Hepatology* 1998;**28**:1607–15.
32 Israel Y. Antibodies against ethanol-derived protein adducts: pathogenic implications. *Gastroenterology* 1997; **113**:353–5.
33 Westendorp RG, Langermans JA, Huizinga TW, *et al.* Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 1997;**349**:170–3.
34 Turner DM, Williams DM, Sankaran D, *et al*. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997;**24**:1–8.
35 Eskdale J, Kube D, Tesch H, *et al.* Mapping of the IL-10 gene and further characterization of the 5' flanking sequence. *Immunogenetics* 1997;**46**:120–8.
36 Lim S, Crawley E, Woo P, *et al.* Haplotype associated with low interleukin-10 production in patients with severe asthma. *Lancet* 1998;**352**:113.
37 Hobbs K, Negri J, Klinnert M, *et al.* Interleukin-10 and transforming growth factor-β promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med* 1998;**158**: 1958–62.
38 Borish L, Aarons A, Rumbyrt J, *et al.* Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol* 1996;**97**:1288–96.
39 Lazarus M, Hajeer AH, Turner D, *et al.* Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. *J Rheumatol* 1997;**24**:2314–17.
40 Edwards-Smith CJ, Jonsson JR, Purdie DM, *et al*. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. *Hepatology* 1999;**30**:526–30.
41 Rosenwasser LJ, Borish L. Genetics of atopy and asthma: the rationale behind promoter-based candidate gene studies (IL-4 and IL-10). *Am J Respir Crit Care Med* 1997;**156**: S152-5.
42 Crawley E, Kay R, Sillibourne J, *et al.* Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum* 1999;**42**:1101–8.
43 Wu A, Slavin G, Levi AJ. Elevated serum gamma-glutamyltransferase (transpeptidase) and histological damage in alcoholics. *Am J Gastroenterol* 1976;**65**:318–23.
44 Grove J, Daly AK, Burt AD, *et al*. Heterozygotes for HFE mutations have no increased risk of advanced alcoholic liver disease. *Gut* 1998;**43**:262–6.
45 Bruguera M, Bordas JM, Rodes J. Asymptomatic liver disease in alcoholics. *Arch Pathol Lab Med* 1977;**101**:644–7.
46 Speilman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus. *Am J Hum Genet* 1993;**52**:506–16.
47 Bashir R, Day CP, James OFW, *et al*. No evidence for involvement of type I collagen structural genes in "genetic predisposition" to alcoholic cirrhosis. *J Hepatol* 1992;**16**: 316–19.
48 Gilligan SB, Cloninger CR. The genetics of alcoholism. In: Crow K, Batt RD, eds. *Human metabolism of alcohol*. Boca Raton, Florida: CRC Press, 1987.
49 Clot P, Parola M, Bellomo G, *et al*. Plasma membrane hydroxyethyl radical adducts cause antibody-dependent cytotoxicity in rat hepatocytes exposed to alcohol. *Gastroenterology* 1997;**113**:265–76.
50 Eskdale J, Gallagher G, Verweij CL, *et al.* Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci USA* 1998;**95**:9465–70.
51 Eskdale J, McNicholl J, Wordsworth P, *et al.* Interleukin-10 microsatellite polymorphisms and IL-10 locus alleles in rheumatoid arthritis susceptibility. *Lancet* 1998;**352**:1282–3.
52 Bogdan C, Vodovotz Y, Nathan C. Macrophage deactivation by interleukin 10. *J Exp Med* 1991;**174**:1549–55.