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IL-6 and IL-10 promoter gene polymorphisms do not associate with the susceptibility for multiple myeloma

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Abstract

Multiple myeloma (MM) is a plasma cell malignancy characterised by bone marrow infiltration and the presence of a monoclonal protein in serum and/or urine. Interleukin-6 (IL-6) has been identified as one of the most important cytokines that contributes to myeloma cell survival and proliferation. Recent investigations suggest involvement of another cytokine, IL-10, in the activation of MM cells. The present study aimed to determine whether there is an association between the polymorphic features located within the promoter regions of IL-6 and IL-10 genes and progression the disease.

IL-6 (−174 G/C) and IL-10 (−1082 A/G, −819 C/T, −592 A/C) promoter single nucleotide polymorphisms (SNPs) were determined by PCR-SSP technique using commercial primers. Our single centre results were compared with the data from literature and combined in cumulative analysis employing the Mantel-Haenszel method.

In univariate analysis, only IL-10 ACC genotype tended to prevail in our (Polish) group of patients. None of IL-6 genotypes or IL-10 (−1082) alleles was found to associate with MM disease either in our single centre or in cumulative study. Among patients who died within 36 months of diagnosis, a significant prevalence ($P < 0.05$) of IL-6 heterozygous cases as opposed to IL-6 homozygotes was observed.

IL-6 and IL-10 promoter gene polymorphisms were not found to associate with the susceptibility to the development of MM. However, the IL-6 polymorphic features appeared as factors that might affect the survival of MM patients. The latter observation warrants further study.

Keywords: IL-6 promoter polymorphism; IL-10 promoter polymorphism; Multiple myeloma

1. Introduction

Multiple myeloma (MM) is a plasma cell malignancy derived from an early precursor of B-cell lineage characterised by a bone marrow infiltration, lytic bone lesions and the presence of a monoclonal protein in serum and/or urine. MM account for 1% of all malignancies in Whites and 2% in US Blacks [1]. MM is rare in patients younger than 30 years and its incidence increases in the elderly. Several cytokines have been implicated in controlling growth, progression and dissemination of MM. Among them the role of interleukin-6 (IL-6) and interleukin-10 (IL-10) is well established [2–5].

IL-6 is a pleiotropic cytokine which plays a role in pathogenesis of various diseases, i.e. inflammation, autoimmune diseases and lymphoid malignancies. This cytokine has been identified as one of the most important cytokines that contributes to myeloma cell survival and proliferation. IL-6 is

Abbreviations: IL, interleukin; MM, multiple myeloma; OR, odds ratio; PCRSSP, polymerase chain reaction with sequence-specific primers; RHM, relative risk calculated by the Mantel-Haenszel method; SNP, single nucleotide polymorphism

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produced by MM cells by an autocrine mechanism, whereas a paracrine mechanism is believed to be involved in the production of IL-6 by the tumour microenvironment in the bone marrow [3,6].

IL-10 is produced by monocytes, T- and B-lymphocytes, natural killer cells and mast cells. IL-10 has been demonstrated to be the most potent B-cell differentiation factor as well as a growth factor for MM cells [7]. It stimulates the proliferation of MM cell lines and of freshly isolated MM cells in short-term bone marrow cultures [7]. Recent investigation suggests the involvement of IL-10 in the activation of MM cells via induction of oncostatin M autocrine loop [8].

High serum levels of both cytokines are observed in MM patients. The serum level of IL-6 is regarded as an important prognostic factor in MM, correlating with the stage of the disease and degree of bone destruction [9]. A higher serum level concentration of IL-10 in MM patients than in the controls as well as the correlation between IL-10 and some clinical and laboratory parameters associated with disease activity has also been observed (Urbanska-Rys et al.). They also analysed relationship between IL-10 and IL-6, and found that higher values of IL-10 in MM patients' serum correlated with IL-6 level [5].

The importance of genetic factors is suggested by the association of an increased risk of developing MM with certain human leukocyte antigens and racial differences in the incidence of MM. Indeed, the MM rates for Blacks in USA are more than double the rates for Whites at 10.8 per 100,000 for Black males and 7.2 per 100,000 for Black females (the comparable figures for American whites are 5.3 per 100,000 for males and 3.3 per 100,000 for females and 0.5 per 100,000 in Hawaiian Japanese males) [10]. A large population-based study found that Black MM patients had significantly higher gene frequencies for Bw65, Cw2 and DRw14, while White MM had higher gene frequencies for A3 and Cw2, and blanks at DR and DQ loci comparison to controls. These findings suggest that the Cw2 allele, or a gene located in proximity to this locus, may confer susceptibility to the development of MM [11]. Reports of plasma and lymphocyte dyscrasias in siblings and other near relatives of MM patients suggest that the predisposition to the development of MM may be inherited [12,13].

Regulation of cytokines secretion has been shown to be under genetic control through genetic polymorphisms in their coding and promoter sequences. The aim of the study was to determine whether there is a prevalence of particular IL-6 and IL-10 genotypes in MM patients.

2. Materials and methods

2.1. Patients and controls

For the study 54 patients (female/male: 31/23) aged 42–88 (median 62) years diagnosed with MM were included. There was only one patient in stage I disease, 14 patients were in stage II and 49 in stage III of the disease (staging according to Durie and Salmon [14]). For patients characteristics, see Table 1. In addition, 50 healthy individuals of both sexes (female/male: 21/29) served as controls.

Table 1
Patients characteristics

Patients (f/m)	54 (31/23)
Age	42–88 (median 62)
Ig subtype	
IgA	10
IgG	31
IgD (one non-secretory)	3
IgM	3
LCD	7
Light chain type	
κ	38
λ	15
Stage	
I	1
II	14
III A/B	23/16
Survival	
Fatal cases/alive	34/20

2.2. IL-6 and IL-10 genotyping

DNA was isolated from the whole blood taken on EDTA with the use of Qiagen DNA Isolation Kit (Qiagen GmbH, Hilden, Germany).

Biallelic polymorphism within the promoter region of IL-6 gene (−174 G/C) and IL-10 gene promoter polymorphism at positions (−1082 A/G, −819 C/T, −592 A/C) were determined by PCR-SSP technique employing commercial primers (One Lambda, Inc., Canoga Park, CA, USA). Please note that the use of this kit (due to a number of primer mix combinations) allow to assess the presence of particular IL-10 haplotypes (i.e. GCC/ATA or ACC/ACC). In brief, for each polymorphic site one PCR reaction was carried out on DNA template with a pair of specific primers, the additional control primes, reaction mix (provided by a manufacturer), and Taq polymerase (Invitrogen, USA) in a total volume of 10 μl. Amplifications were performed in MJ Research Apparatus (Watertown, MA, USA). PCR cycling conditions were as follows: 96 °C for 130 s, 63 °C for 60 s, followed by nine cycles of 96 °C for 10 s, 63 °C for 60 s, and followed by 20 cycles of 96 °C for 10 s, 59 °C for 50 s, 72 °C for 30 s, ending with 4 °C. PCR products were analysed electrophoretically in 2% agarose gel and visualised under UV.

2.3. Statistical analysis

Genotype and allele frequencies were compared between the study groups by the χ^2-test with Yates correction or Fisher's exact test when necessary. The odd's ratio (OR) was