−174 G>C Polymorphism of Interleukin 6 Gene Promoter Affects Interleukin 6 Serum Level in Patients with Colorectal Cancer

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ABSTRACT

Purpose: Experimental data suggest that interleukin 6 (IL-6) plays an important role in the development and progression of metastasis from colorectal cancer (CRC), and −174 G>C polymorphism has been identified recently in the IL-6 gene promoter. Therefore, the aim of the present study was to investigate the significance of this type of polymorphism in patients with CRC.

Experimental Design: Using enzyme immunoassay, IL-6 concentrations were measured in preoperative serum samples from 65 stage I-IV CRC patients. DNA was extracted from peripheral blood mononuclear cells, and −174 G>C polymorphism detected using PCR, followed by NlaIII restriction enzyme digestion and electrophoresis.

Results: The median IL-6 serum level was 0.14 pg/ml in patients with stage I-III disease versus 0.41 pg/ml in patients with stage IV disease (P < 0.001). DNA amplification was possible in 62 cases. On grouping genotypes at the −174 G>C locus as C+ (CC and CG) and C− (GG), a significant association was observed between the type of polymorphism and IL-6 serum level: the median value for IL-6 was 0.14 pg/ml in C+ patients (n = 32) and 0.32 pg/ml in C− patients (n = 30; P = 0.034). Moreover, in patients with hepatic metastasis the median level of IL-6 was 0.23 pg/ml in C+ patients (n = 9) and 0.96 pg/ml in C− patients (n = 9; P = 0.004).

Conclusions: In patients with CRC, the −174 G>C polymorphism status of the IL-6 gene promoter affects the IL-6 serum level, particularly in the presence of hepatic metastasis.

INTRODUCTION

IL-6,² a M, 25,000 glycoprotein growth factor, has multiple stimulatory effects on inflammation and cell growth (1). Experimental findings suggest that IL-6 acts as a potent stimulator of metastasis by up-regulating the expression on endothelial cells of adhesion receptors, such as intercellular adhesion molecule-1 and leukocyte adhesion molecule-1, and by stimulating the production of growth factors such as hepatocyte growth factor and vascular endothelial growth factor (2–4). Several clinical studies have reported that in different tumor types, a high IL-6 serum level is associated with advanced stage disease (5–8) and a worse outcome (5, 9, 10). In particular, we reported elsewhere that a high preoperative IL-6 serum level is associated with the presence of metastasis and is a negative prognostic factor in patients with CRC (11).

The status of a common functional single G>C base exchange polymorphism in the human IL-6 gene promoter (chromosome 7p21) located at 174 bp, upstream from the start site of transcription (−174 G>C locus) (12), has been reported to influence IL-6 levels in vitro and in vivo (13, 14). The G allele increases IL-6 expression, both in basal and stimulated conditions, the highest IL-6 levels in plasma and serum being found in subjects homozygous for the G allele (13, 14). Moreover, it has been reported that −174 G>C polymorphism is involved in several chronic conditions, such as insulin and noninsulin-dependent diabetes mellitus, juvenile chronic arthritis, coronary heart disease, dementia, peripheral artery occlusive disease, and postmenopausal osteoporosis (14–21). However, to our knowledge, no studies have been conducted to evaluate the effect of −174 G>C polymorphism on IL-6 serum levels in patients with solid tumors. Therefore, the aim of the present study was to investigate the influence of −174 G>C polymorphism on IL-6 serum levels in patients with CRC, particularly those with hepatic metastasis.

PATIENTS AND METHODS

Patients. The study population consisted of 65 fully informed consent patients (42 men and 23 women; mean age 66 years; range, 43–83 years), who underwent surgery for colorectal adenocarcinoma at our institution between January 2001 and December 2001. Patients with hereditary CRC, inflammatory bowel disease, and obstructing or perforated tumor were excluded from the study. None of the patients underwent preoperative chemoradiotherapy. The tumor was located in the colon in 47 cases (72%) and in the rectum in 18 cases (28%). The histological grade was assessed according to WHO criteria (22): 13 tumors (20%) were well differentiated, 34 (53%) mod-

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²The abbreviations used are: IL, interleukin; CRC, colorectal cancer; C, cytosine; G, guanine; TNM, tumor-node-metastasis; NF, nuclear factor.
erately differentiated, and 18 (27%) poorly differentiated. Following the International Union Against Cancer classification and TNM staging system (23), 20 of the tumors (31%) were stage I, 14 (21%) stage II, 13 (20%) stage III, and 18 (28%) stage IV. All of the patients with stage IV disease had hepatic metastasis, which involved <50% of the liver in 10 patients (56%) and >50% of the liver in 8 patients (44%). Four of the patients with hepatic metastasis (22%) underwent resection, 8 (45%) systemic chemotherapy, 2 (11%) locoregional treatment, and 4 (22%) were treated by supportive care only.

Blood samples were obtained from fasting patients immediately before anesthesia and surgery.

**Serum IL-6 Levels.** Blood, collected in pyrogen-free glass tubes, was centrifuged at 600 rpm for 10 min. The serum was removed and stored at −80°C in pyrogen-free plastic tubes until analysis. Serum IL-6 levels were measured using a commercially available dual antibody sandwich enzyme-linked immunoassay (BioSource Cytoscreen human IL-6 UltraSensitive kit; Biosource International, Camarillo, CA). Using this kit, which has been used in previous studies (13, 24), the minimum detectable dose of IL-6 was 0.10 pg/ml, and no cross-detection of other cytokines was observed. All of the samples were thawed only once and assayed in duplicate. The intra-assay coefficient of variation was <10%.

**Determination of −174 G>C Polymorphism.** DNA, extracted from peripheral blood mononuclear cells using phenol-chloroform following standard procedures, was amplified by primers designed for the promoter region of the IL-6 gene, as described previously (12). The primers used were: TTG TCA AGA CAT GCC AAG TGC T (forward) and GCC TCA GAG ACA TCT CCA GTC C (reverse).

PCR products were digested with the NlaIII restriction enzyme overnight and electrophoresed on 2% agarose gel. The presence of a cytosine (C allele) at nucleotide −174 was revealed by the presence of the NlaIII cutting site.

The genotypes identified at the −174 G>C locus were classified as G/G (homozygotes for the NlaIII cutting site absence), G/C (heterozygotes for the NlaIII cutting site absence and presence), and C/C (homozygotes for the NlaIII cutting site presence). On the basis of our previous experience (13), genotypes were subsequently grouped as C+ (CC and CG) and C− (GG).

**Statistical Analysis.** Because of the great departure from the normality of log-transformed data, a comparison was made between serum IL-6 levels in relation to genotype and clinicopathologic variables using the Mann-Whitney or Kruskall-Wallis nonparametric rank tests (25). Ps < 0.05 were considered significant. The data are reported as medians with the first and the third quartiles, the latter representing variability within each group. All of the analyses were computed with S-Plus software (StatInc., Seattle, WA).

**RESULTS**

**Correlation between IL-6 Serum Level and Clinical-Pathologic Variables.** Serum IL-6 levels were not significantly correlated with sex, age, or tumor site and grade. However, in patients with stage I-III disease (n = 47) the median IL-6 serum level was 0.14 pg/ml (0.24–2.34), whereas in patients with hepatic metastasis (n = 18) the median IL-6 serum level was 0.41 pg/ml (0.10–0.37; P < 0.001). No statistically significant difference was found between the IL-6 serum level of patients with stage I-II and that of patients with stage III disease (Table 1).

**Correlation among −174 G>C Polymorphism, IL-6 Serum Level, and Clinicopathologic Variables.** The impact of the −174 G>C locus on the IL-6 serum level was evaluated in 62 patients (in 3 cases DNA amplification was not possible), of whom 4 were C/C, 28 C/G, and 30 G/G. The genotype distribution among patients was in Hardy-Weinberg equilibrium (P > 0.05). The median IL-6 serum levels in the three genotypes were: 0.33 pg/ml (0.15–0.42) in C/C, 0.14 pg/ml (0.10–0.22) in C/G, and 0.32 pg/ml (0.10–0.94) in G/G. On comparing the homozygous group C/C versus G/G, and C/C versus C/G and G/G, no statistically significant difference was found for IL-6 levels; this was probably because of the low number of C/C patients. On the other hand, a significant association was observed between C+/C− status and IL-6 serum level: the median IL-6 serum level was 0.14 pg/ml (0.10–0.28) in C+ patients (n = 32) and 0.32 pg/ml (0.10–0.94) in C− patients (n = 30; P = 0.034). No significant association was found between genotype and IL-6 serum level when patients were stratified for sex, age, site, grade, and stage I-III. As shown in Table 2, a strong correlation was found between the genotype and the IL-6 serum level in patients with hepatic metastasis: the median IL-6 serum level was 0.23 pg/ml (0.15–0.30) in C+ patients (n = 9) and 0.96 pg/ml (0.45–6.62) in C− patients (n = 9; P = 0.004).

**DISCUSSION**

Our findings demonstrate that −174 G>C polymorphism in the IL-6 gene promoter influences the levels of circulating IL-6 in patients with CRC, and to our knowledge, are the first
metastasis may depend on IL-6 being a potent stimulator of tumoral IL-6 originating in tumor infiltrating mononuclear cells of CRC patients, and found specific translation activation of IL-6 by NFκB. This transcription factor, known to regulate IL-6 transcription, is reportedly overexpressed in highly metastatic cell lines as well as in metastatic tissue (32, 33). IL-6 promoter activation involves synergism between the transcription factors NF-IL-6 and NFκB (34), and this may explain why we found that IL-6 serum levels varied significantly in the subset of patients with hepatic metastasis, depending on the −174 G>C polymorphism status.

In conclusion, our findings indicate that −174 G>C polymorphism influences circulating levels of IL-6 in patients with CRC. This observation may in fact be of clinical relevance, because high serum IL-6 level has been shown to be a negative prognostic factor in patients with several tumor types, including CRC (5, 9–11). As this genetic variant may be a prognostic molecular marker, it should be additionally investigated in prospective studies.

ACKNOWLEDGMENTS

We thank Sara Pearcey for correcting the English language.

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