Relationship Between Interleukin 6 Promoter Polymorphism at Position –174, IL-6 Serum Levels, and the Risk of Relapse/Recurrence in Polymyalgia Rheumatica

LUIGI BOIARDI, BRUNO CASALI, ENRICO FARNETTI, NICOLÒ PIPITONE, DAVIDE NICOLI, FABRIZIO CANTINI, PIERLUIGI MACCHIONI, GIANLUIGI BAJOCCHI, MARIA GRAZIA CATANOSO, LIA PULSATELLI, DARIO CONSONNI, and CARLO SALVARANI

ABSTRACT. ***Objective***. To assess the role of –174 G/C promoter polymorphism of interleukin 6 (IL-6) in the susceptibility to polymyalgia rheumatica (PMR). We also investigated whether this polymorphism modulates the circulating level of IL-6 and the risk of relapse/recurrence in a series of patients with PMR followed up prospectively.

Methods. A prospective study of 112 consecutive, untreated patients with isolated PMR (i.e., without evidence of giant cell arteritis) who were followed up for at least 24 months. This cohort represented all patients diagnosed over a 5-year period in one Italian rheumatological secondary referral center. Patients were monitored for clinical signs/symptoms and acute-phase reactants. All PMR patients and 112 population-based controls from the same geographic area were genotyped for IL-6 polymorphism at position –174 by molecular methods. IL-6 serum levels were measured in 67 PMR patients and 43 population-based controls.

Results. The distribution of the G/C 174 genotype was similar in PMR patients and controls. No significant associations with IL-6 promoter polymorphism at position –174 were found when PMR patients with and without relapse/recurrence were compared. Controls homozygous for the C allele had higher serum IL-6 levels than the carriers of the G allele (4.5 ± 3.7 pg/ml vs 1.8 ± 2.1 pg/ml, p = 0.01). Patients homozygous for the allele C had significantly higher values of IL-6 during followup than patients carrying GC or GG genotypes. CC homozygosity was significantly more frequent in patients with persistently elevated levels of IL-6 than in those without. The presence of persistently elevated IL-6 levels, but not the CC genotype, was associated with an increased frequency of relapse/recurrence.

Conclusion. Our findings show that the 174 G/C promoter IL-6 polymorphism is not implicated in susceptibility to PMR. However, CC genotype characterized PMR patients with persistently elevated levels of IL-6 who are at higher risk of developing relapse/recurrence. A genetically modulated pattern of IL-6 production could affect the longterm outcome of patients with PMR. (J Rheumatol 2006;33:703–8)

Key Indexing Terms:
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Interleukin 6 (IL-6) is the chief stimulator of the production of most acute-phase proteins[1]. Increased production of IL-6 is a characteristic finding in patients with polymyalgia rheumatica (PMR), and corticosteroids (CS) rapidly reduce the levels of circulating IL-6. IL-6 seems to be a sensitive indicator of disease activity in PMR[2-6]. Some studies have demonstrated that persistently elevated levels of IL-6 characterized PMR patients at a higher risk of developing relapse/recurrence and who required higher initial CS dose and prolonged CS therapy[5-6]. Recently, a bi-allelic polymorphism, localized at position –174 of the promoter region of the IL-6 gene, has been identified. This polymorphism has been reported as functionally important, since it influences the transcription rate of the gene and plasma concentrations of IL-6[7].

We assessed the role of this IL-6 polymorphism in the susceptibility to, and severity of, PMR. In particular, we investi-

From the Servizio di Reumatologia and Laboratorio di Biologia Molecolare, Arcispedale S. Maria Nuova, Reggio Emilia; Unità Reumatologica, Divisione di Medicina Interna, Ospedale di Prato, Prato; Laboratorio di Immunologia e Genetica, Istituti Ortopedici Rizzoli, Bologna; Azienda Ospedaliera Istituti Clinici Perfezionamento, Italy.

L. Boiardi, MD, PhD, Servizio di Reumatologia; B. Casali, MD; E. Farnetti, BD, Laboratorio di Biologia Molecolare, Milano; N. Pipitone, MD, PhD, Servizio di Reumatologia; D. Nicoli, BD, Laboratorio di Biologia Molecolare, Arcispedale S. Maria Nuova; F. Cantini, MD, Unità Reumatologica, Divisione di Medicina Interna, Ospedale di Prato; P. Macchioni, MD; G. Bajocchi, MD; M. Grazia Catanoso, MD, Servizio di Reumatologia, Arcispedale S. Maria Nuova; L. Pulsatelli, BD, Laboratorio di Immunologia e Genetica, Istituti Ortopedici Rizzoli; D. Consonni, MD, PhD, Azienda Ospedaliera Istituti Clinici Perfezionamento; C. Salvarani, MD, Servizio di Reumatologia, Arcispedale S. Maria Nuova.

Address reprint requests to Dr. C. Salvarani, Servizio di Reumatologia, Arcispedale S. Maria Nuova, V. le Risorgimento N80, 42100 Reggio Emilia, Italy. E-mail: salvarani.carlo@asmn.re.it

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gated whether the –174 G/C promoter polymorphism of IL-6 might modulate the circulating level of IL-6 and the risk of relapse/recurrence in a series of patients with PMR followed up prospectively.

MATERIALS AND METHODS

Study population. One hundred-twenty consecutive, untreated patients with pure PMR, representing all the patients diagnosed over a 5-year period (1993–1997) in one Italian rheumatology secondary referral center (Reggio Emilia) were studied. One hundred twelve patients were followed up for at least 2 years.

The diagnosis of PMR was confirmed if all the following criteria were met[8]: (1) persistent pain (for at least one month) involving at least 2 of the following areas: neck, shoulders, and/or pelvic girdle; (2) early morning stiffness lasting longer than one hour; (3) rapid response to prednisone (or equivalent) (≥ 20 mg/day); (4) age greater than 50 years; (5) absence of other diseases capable of causing musculoskeletal symptoms consistent with PMR. Patients with erythrocyte sedimentation rate (ESR) < 40 mm/h but who satisfied other criteria were included in the study.

All patients were seronegative for rheumatoid factor. At diagnosis and during followup, all patients were evaluated using the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for rheumatoid arthritis (RA)[9]. Those who satisfied these criteria and those with clinical manifestations of giant cell arteritis (GCA) or positive temporal artery biopsy were excluded.

Patients were clinically assessed by the same rheumatologist at study onset, once monthly for the first 6 months, and then every 3 months during the followup period. A standardized data collection form was used at every visit to record medical information including age, sex, site(s) of aching and morning stiffness, presence of systemic manifestations (defined by the presence of asthenia, anorexia, weight loss, and/or fever), dosage and duration of CS therapy, and development of relapse and recurrence. The presence or absence of swelling and tenderness of the joints and periarticular structures was carefully assessed at each visit.

All patients were treated with prednisone starting with a median dose of 12.5 mg daily (range: 10–25 mg/daily) as a single morning dose. If there was no clinical improvement, the initial dose was increased by 2.5–5 mg/day before the tapering schedule was started. Prednisone dosage was reduced in all patients according to the same fixed schedule, starting one month after symptoms had resolved. Small monthly decrements of 5 mg to 1 mg were subsequently implemented until the minimal maintenance dose of 2.5 mg every other day was reached. CS were withdrawn after one month if both clinical and humoral (ESR < 30 mm/h and CRP < 0.5 mg/dl) remission persisted.

Joint radiography was performed in all patients with joint swelling at some point during the course of the illness. Relapse and recurrence were defined as reappearance of symptoms associated with elevated ESR and/or C-reactive protein (CRP) (ESR > 30 mm/h and/or CRP > 0.5 mg/dl) in patients receiving CS and after discontinuation of treatment, respectively. The symptoms were suppressed by resumption of, or increase in, prednisone dose.

The end of the disease was recorded as the date of permanent discontinuation of therapy without subsequent relapse or recurrence. The endpoint of patient followup was the date of the last visit or the date of death. Over the followup period no articular deformities and/or radiological evidence of erosions were found in any of the 112 patients. In addition, none of them developed clinical features that satisfied the 1987 modified ACR criteria for RA[9].

Control subjects were randomly recruited from the lists of patients who were under the care of the same public health service medical practitioners. Stratification by random number of the group by age and sex was used to approximately match the controls with the cases according to age and sex distribution. At the end of this selection process, 112 control subjects were identified. The median age of the controls was 69 years (range: 50–79). Of controls 27.8% were male and 72.2% female. All study subjects were white, of Italian descent, and had been resident in Italy for at least one generation. No ethnic differences were found between the patients and the controls. No study participant had a Jewish background.

The study was approved by the Ethics Committee of Reggio Emilia Hospital, and informed consent was obtained from all patients or their relatives.

Laboratory analysis. Laboratory tests were performed on blood samples drawn in the morning before daily administration of the scheduled prednisone dose. Serial blood samples were collected at the same time of the clinical evaluation at therapy onset, at monthly intervals for the first 6 months, and subsequently at 3-monthly intervals until the end of followup. ESR was determined using the Westergren method. CRP was measured by nephelometry (NA latex CRP kit, Behringwerke, Marburg, Germany; upper limit of the normal reference range 0.5 mg/dl). Serum IL-6 concentrations were evaluated by immunoassay using a commercial ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The sensitivity of the test is 0.70 pg/ml. IL-6 serum levels were measured in 66 PMR patients at baseline and during the followup and in 43 controls matched with PMR patients for age and sex. Normal IL-6 levels were considered < 4 pg/ml (normal mean + 3 SD).

DNA extraction and genotyping. DNA was obtained from whole blood using phenol/chloroform method, according to standard procedures. We designed a new primer pair to amplify a 268 bp region of IL-6 promoter containing –174 G/C polymorphism (forward primer 5' TTC GTG CAT GAC TTC AGC TT 3' and reverse primer 5' ACT CAT GGG AAA ATC CCA CA 3')[7]. PCR amplification was performed in 25 μl reaction containing 100 μM of each dNTP, 20 pmol each primer, 1 unit Taq polymerase. Amplification profile was as follows: initial denaturation 95°C for 2 min, 35 cycles of: 94°C for 30 s, 62°C for 30 s, 72°C for 30 s, and final extension at 72°C for 3 min.

We performed digestion of 10 μl PCR product using Nla III restriction endonuclease. This enzyme can reveal the presence of C or G nucleotide at –174 position after electophoresis analysis of digested PCR products in 2% agarose gel stained with ethidium bromide (0.5 μg/ml).

Statistical analysis. Statistical analysis was done using SPSS and SAS statistical packages (SPSS Inc., Chicago, IL; SAS Institute Inc., Cary, NC, USA). Student's t test and Mann-Whitney test were computed to compare means for parametrically and non-parametrically distributed data, respectively. The frequencies of the alleles and genotypes among the case patients and control group were compared by chi-square test or by Fisher exact test, whichever was appropriate. Odds ratios (OR) were calculated together with their 95% confidence intervals (95% CI). Corrected p values were calculated by multiplying p by the number of alleles compared. Cumulative probability of not having relapse/recurrence was analyzed with the Kaplan-Meier method and compared by means of the log-rank test. IL-6 and CRP values obtained at different times after the beginning of therapy during the 2-year followup period were pooled in one average value. Data were analyzed by a 2-way analysis of variance considering as factors carriage rate (CC, CG+GG) and treatment (before or after the beginning of therapy) using the GLM procedure of SAS. We performed power calculation for an unmatched case-control study and estimated the relative risk using Power and Sample Size software, version 2.1.31.

RESULTS

Table 1 shows the clinical and demographic characteristics of the 112 patients with isolated PMR. The allele and genotype frequencies of the –174 G/C promoter polymorphism of IL-6 were not statistically different in PMR patients versus controls as shown in Table 2. Given the sample sizes (120 cases with PMR and 112 controls) and the allele frequencies of the polymorphism examined, we can exclude with 80% certainty a genetic relative risk of 1.5 at –174 G/C IL-6 promoter polymorphism. Kaplan-Meier analysis revealed no differences in the cumulative probability of not having relapse/recurrence during the followup among the 3 different G/C 174 genotypes

Table 1. Demographic, clinical and laboratory findings of the 112 patients with polymyalgia rheumatica followed for at least 2 years. Values are mean ± SD unless otherwise indicated.

Male/female, %	26.8/73.2
Age at onset of disease, yrs	72 ± 7
Duration of symptoms before diagnosis, mo	3 ± 1
Duration of therapy, mo	30 ± 22
Duration of followup, mo	51 ± 25
Systemic symptoms and/or signs (fever, anorexia, weight loss), %	45
Morning stiffness at diagnosis, h	3 ± 1
Peripheral arthritis, %	23
Patients with at least 1 relapse/recurrence, No. (%)	49 (43.7)
Patients with at least 2 relapses/recurrences, No. (%)	26 (23.2)
Starting prednisone dose, mg/day	18 ± 8
ESR at diagnosis, mm/h	74 ± 28
CRP at diagnosis, mg/dl	6 ± 4
IL-6 at diagnosis, pg/ml*	26 ± 25

* IL-6 serum levels were determined in 66 patients.

Table 2. Frequency of alleles, genotypes, and carriage rates of IL-6 promoter polymorphism at position –174 in patients with polymyalgia rheumatica and controls.

Variable	Controls (n = 112) (%)	PMR (n = 120) (%)	p
Alleles			
C	83/224 (37.1)	84/240 (35.0)	NS
G	141/224 (62.9)	156/240 (65.0)	
Genotypes			
CC	16/112 (14.3)	16/120 (13.3)	
CG	51/112 (45.5)	52/120 (43.3)	NS
GG	45/112 (40.2)	52/120 (43.3)	
Carriage rate			
CC + CG	67/112 (59.8)	60/120 (56.7)	NS
GG	45/112 (40.2)	52/120 (43.3)	
CG + GG	96/112 (85.7)	104/120 (86.7)	NS
CC	16/112 (14.3)	16/120 (13.3)	

(log-rank test, p = 0.71) (Figure 1). Table 3 shows the effect of carriage rate (CC, GC+GG) and treatment on IL-6 and CRP values during followup. No interaction between carriage rate and time was found. Patients homozygous for the C allele had significantly higher values of IL-6 than patients carrying GC or GG genotypes (p = 0.037). CRP serum values were not affected by carriage rate. As expected prednisone treatment significantly reduced both IL-6 and CRP values (p < 0.001).

Table 4 shows the frequencies of alleles, genotypes, and carriage rates of the –174 G/C polymorphism in patients with and without elevated levels of IL-6 at all time points during the 2-year followup period. CC homozygosity was significantly more frequent in patients with persistently elevated levels of IL-6 than in those without [p = 0.002, pcorr = 0.004, OR 34.4 (95% CI: 3.2–369.8)]. However, the analysis was limited by the low number of patients evaluated. All 4 patients homozygous for the C allele and with persistently elevated levels of IL-6 had at least one relapse/recurrence (median: 3, range: 1-4), while only 1 of the 5 patients homozygous for the C allele but without persistently elevated levels of IL-6 had at least one relapse/recurrence (100% vs 20%, p = 0.048).

The 33 genotyped controls also had serum IL-6 levels measured. The 6 patients homozygous for the C allele had higher serum IL-6 levels than the 27 carriers of the G allele (GG+CG) (4.5 ± 3.7 pg/ml versus 1.8 ± 2.1 pg/ml, p = 0.01).

DISCUSSION

The –174 G/C promoter polymorphism of IL-6 has been associated with a variety of diseases, such as Alzheimer disease, cardiovascular disease, cancer, diabetes, osteoporosis, sepsis, and systemic-onset juvenile chronic arthritis[7,10-17]. In our study, we did not observe an association between –174 G/C promoter polymorphism of IL-6 and isolated PMR. This absence of association with this IL-6 polymorphism is consistent with the observations of Gonzalez-Gay, *et al*, who showed an association of the C allele with the development of PMR features in GCA but not with isolated PMR[18].

We also examined whether –174 G/C promoter polymorphism was associated with PMR severity by prospectively following up our patient cohort for at least 2 years. The course of PMR is heterogeneous with a variable CS dose requirement and therapy duration. A treatment course of one to 2 years is often required[19]. However, about 30% to 50% of the patients have a remitting-relapsing course and thus require longterm CS treatment (> 2 yrs)[20-24]. Some studies have suggested that genetic makeup may contribute to PMR severity. HLA-DR4, HLA-DR1, and G/R 241 polymorphisms of the intercellular adhesion molecule 1 (ICAM-1) confer an increased risk of relapse/recurrence in patients with PMR[25-29]. In Northwestern Spain ICAM-1 polymorphisms alone did not appear to be associated with disease severity in isolated PMR; however, the presence of both HLA-DRB1*0401 and ICAM-1 codon 241 GG homozygosity was significantly associated with an increased risk of relapses in these patients[30].

No association between –174 G/C promoter polymorphism and a remitting-relapsing course of PMR was found in our study. These results are again in line with the observations of Gonzalez-Gay *et al*, who found no associations between this polymorphism and disease severity in Spanish patients with PMR[18].

IL-6 appears to be a potentially useful biologic marker of disease activity in PMR. IL-6 is the chief stimulator of production of most acute-phase proteins, including CRP[1]. Increased production of IL-6 is a characteristic finding in patients with PMR, while CS therapy leads to an abrupt decline in circulating IL-6 levels, which closely correlates with the remission of clinical symptoms[2-6]. Two studies showed that persistently elevated levels of CRP after one week of treatment [31] and of IL-6 after one month[5] characterized PMR patients who required higher initial CS dose and prolonged CS therapy. Recently, we provided evidence that

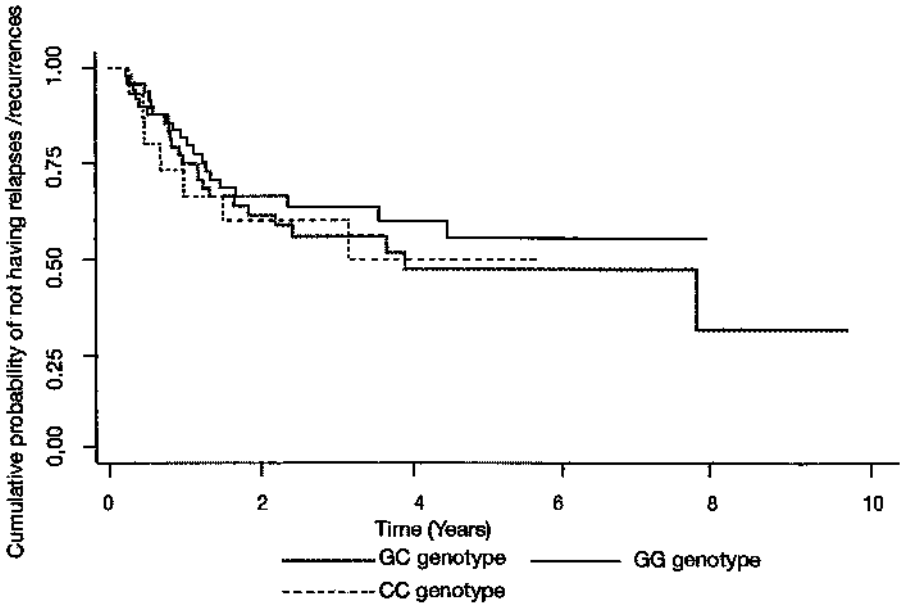

Figure 1. Cumulative probability of not having relapse/recurrence during the followup according to IL-6 genotype. No differences were observed among the 3 different genotypes (log-rank test, p = 0.71).

Table 3. IL-6 and CRP least-squares means according to carriage rate and treatment. Values are mean ± standard error.

	CC	GC + GG	p	Baseline	After Starting Prednisone	p
IL-6, pg/ml	27.1 ± 5.9	13.8 ± 2.3	0.038	25.0 ± 3.7	11.9 ± 5.1	< 0.001
CRP, mg/dl	2.9 ± 0.7	3.3 ± 0.3	0.6	5.47 ± 0.4	0.8 ± 0.6	< 0.001

p values are according to type III test of fixed effects.

Table 4. Frequencies of alleles, genotypes and carriage rate of the –174 G/C promoter polymorphism of IL-6 in patients with and without persistently elevated levels of IL-6 during the 2-year followup period*. Values in parentheses are percentages.

Variable	Patients With Persistently Elevated Levels, n = 5	Patients Without Persistently Elevated Levels, n = 48	p
Alleles			
C	9/10 (90.0)	34/96 (35.4)	0.001
G	1/10 (10.0)	62/96 (64.6)	p corr: 0.002
Genotypes			
CC	4/5 (80.0)	5/48 (10.4)	0.001
CG	1/5 (20.0)	24/48 (50.0)	p corr: 0.003
GG	0/5 (0)	19/48 (39.6)	
Carriage Rate			
CC	4/5 (80.0)	5/48 (10.4)	0.002
CG + GG	1/5 (20.0)	43/48 (89.6)	p corr: 0.004

** IL-6 serum levels were determined in 53 patients with a followup period of 2 years.

despite the apparent control of clinical symptoms, CS do not adequately control the inflammation in a subset of patients with PMR characterized by persistently elevated level of CRP and IL-6 and by a higher risk of developing relapse/recurrence[6].

Roche, *et al* demonstrated that administration of CS rapidly reduced the levels of circulating IL-6 and argued that CS may not be able to correct the underlying mechanism(s) inducing the increased IL-6 production[4]. In individual patients, short term withdrawal of CS, even after several months of treatment, was followed by an immediate increase in circulating IL-6 concentrations in parallel with the reappearance of symptoms. Therefore, the authors hypothesized that the proinflammatory mechanism(s) persist for a prolonged period in the patients with PMR and GCA. Consistent with this consideration, CS would not be able to switch off a genetically regulated hyperproduction of IL-6 related to chronic inflammatory stimulation in the subset of PMR patients with relapse/recurrence.

Although studies investigating the role of the –174 G/C promoter polymorphism in regulating plasma IL-6 concentrations *in vivo* have produced conflicting results[7,10,12,32-41], the –174C allele has been found to be associated with raised IL-6 levels in patients with abdominal aortic aneurysms[32]. Further, in patients homozygous for the –174 C allele, plasma concentrations of IL-6 induced by coronary artery bypass grafting, were significantly higher 6 h after surgery[33]. In our study, there was a significant effect of genotype on IL-6 levels in the controls, confirming that CC homozygotes have higher IL-6 levels than the carrier of the G allele. We also evaluated serum concentrations of IL-6 and CRP at the time of diagnosis and at different time points (pooled in one average value) during the 24-month followup period stratified according to the carriage rate of the –174 G/C promoter polymorphism of IL-6. Patients homozygous for the C allele had significantly higher values of IL-6 than patients carrying GC or GG genotypes. Further, CC genotype was significantly associated with the presence of persistently elevated IL-6 levels. The presence of persistently elevated IL-6 levels, but not the CC genotype, was associated with an increased frequency of relapse/recurrence. A suggested model is that a constant chronic inflammatory stimulus may induce in PMR patients genotypically determined, persistently increased production of IL-6 that may affect the longterm outcome of these patients.

In conclusion, our study confirms that the 174 G/C promoter IL-6 polymorphism does not appear to be implicated in the susceptibility to developing isolated PMR. We observed in control subjects an association between CC genotype and higher levels of circulating IL-6. This association was also present in PMR patients, in particular, this genotype characterized the subgroup of patients with persistently elevated levels of IL-6 that had a higher risk of developing relapse/recurrence. A genetically modulated pattern of IL-6 production could affect the longterm outcome of PMR patients. However, due to the limited power of our study, in order to confirm our data multicenter collaborations to recruit an adequate number of patients are required.

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