

C(−260)→T Polymorphism in the Promoter of the CD14 Monocyte Receptor Gene as a Risk Factor for Myocardial Infarction

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Background—The CD14 receptor of monocytes is an important mediator for the activation of monocytes/macrophages by endotoxins from the envelope of Gram-negative bacteria (lipopolysaccharides). We identified a polymorphism in the CD14 receptor and examined whether this genetic marker influenced the expression of the CD14 receptor on monocytes and affected the predisposition to myocardial infarction.

Methods and Results—We identified a C(−260)→T nucleotide change, creating a *Hae*III polymorphism in the promoter of the CD14 gene. The polymorphism was determined in 178 male patients <65 years old (cases; average age, 55.9±6.3 years) at the time of their first myocardial infarction and in 135 representative selected male control subjects (controls; average age, 55.2±11.5 years). The frequency of the T allele (absence of the cutting site) was 0.49 in cases and 0.35 in controls ($P=0.0005$; OR, 1.781; 95% CI, 1.286 to 2.465). Subsequently, we measured the expression of monocyte CD14 by flow cytometry in 18 volunteers with different CD14 genotypes. A significantly higher density of the CD14 receptor was shown in the T/T homozygotes than in the others ($P=0.0028$).

Conclusions—A higher frequency of allele T(−260) in the promoter of the CD14 receptor gene was found in myocardial infarction survivors than in controls. At the same time, this variation was associated with a higher density of CD14 receptors in healthy volunteers. Therefore, we can conclude that in addition to the well-established risk factors, a genetically determined reaction of monocytes/macrophages to infectious stimuli could play an important role in the process of atherosclerosis. (*Circulation*. 1999;99:3218-3220.)

Key Words: genetics ■ myocardial infarction ■ epidemiology ■ immune system

Recently published studies^{1,2} have suggested that in addition to the well-established risk factors, inflammatory and infectious processes may participate in the initiation of atherosclerosis and in its complications. The attachment of monocytes to the endothelium, followed by their migration into the intima, is one of the first and most crucial steps in the development of atherosclerotic lesions. In the subendothelial space, these highly active cells mature into tissue macrophages and synthesize proinflammatory and prothrombotic substances.

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Among other factors, monocyte maturation could be influenced by the lipopolysaccharides (LPSs, endotoxins of Gram-negative bacteria). LPS is internalized by cells through the CD14 receptor.³ The CD14 receptor is a glycoprotein localized on the cell surface of all myeloid cells, especially on monocytes/macrophages.⁴ The stimulation of monocytes/macrophages by LPS induces overexpression of certain cytokines, complement components, coagulation factors, and many others.^{3,5}

The gene for the CD14 receptor consists of ≈3900 bp organized in 2 exons and encodes a protein of 375 amino acids.⁶ The promoter of the gene has been sequenced and characterized, and an Sp1 transcription factor binding site was identified as critical for CD14 expression.⁷ We identified a polymorphism localized near the binding site for Sp1 and examined its possible influence on the risk of myocardial infarction (MI) as well as on the expression of the CD14 receptor.

Methods

Subjects

Case patients were 178 men <65 years old (average age, 55.9±6.3 years) who had survived their first MI and were admitted to 1 of 2 coronary care units within a 22-month period. The blood samples for genetic and biochemical analysis were obtained within 72 hours after admission to coronary care units. The control group represented a 1% population sample of the adult men (average age, 55.2±11.5 years) randomly selected in 1 Czech district according to the protocol of the MONICA study.⁸ The approval of the local ethics board and informed consent of the participants were obtained before the study.

Received February 17, 1999; revision received May 4, 1999; accepted May 5, 1999.

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Allele and Genotype Frequencies of the C(−260)→T Polymorphism in the CD14 Gene Promoter in Patients With Myocardial Infarction and in the Control Group

	Patients (n=178)		Controls (n=135)		P
	n	%	n	%	
Allele					
T	175	49.2	95	35.2	0.0005
C	181	50.8	175	64.8	
Genotype					
T/T	49	27.5	21	15.6	0.0049
C/T	77	43.3	53	39.3	
C/C	52	29.2	61	45.2	

To avoid the possible influence of genetic nonhomogeneity of the samples,⁹ the control subjects and the patients were selected from the same geographic region of the Czech republic.

In addition, 5 T/T homozygotes, 5 C/T heterozygotes, and 8 C/C homozygotes were selected from 38 young (20- to 30-year-old) male volunteers for monocyte CD14 receptor density determination.

Biochemical Analysis

The lipoprotein parameters were measured enzymatically by the WHO Lipid Reference Center on a Roche COBAS MIRA autoanalyzer (Hoffmann–La Roche) with reagents from Boehringer Mannheim Diagnostics and Hoffmann–La Roche.

The density of the monocyte CD14 receptors was measured by flow cytometry with a monoclonal antibody (clone Mφ P9) according to the standard protocol.¹⁰

DNA Analysis

DNA was isolated by a standard method.¹¹ Polymerase chain reaction (PCR) was performed at a total volume of 50 μ L (100 to 200 ng of genomic DNA, 1 U *Taq* DNA polymerase, 50 pmol of each primer, 200 nmol of each dNTP, and 1.5 mmol Mg^{2+}). The promoter of the CD14 receptor gene was amplified by the primers CDP-1, 5'-TTGGTGCCAAACAGATGAGGTTTAC, and CDP-2, 5'-TTCTTTCCTACACAGCGGCACCC on an OmniGene Thermocycler (Hybaid) under the following conditions: an initial denaturation at 95°C for 2 minutes, followed by 35 cycles at 92.3°C for 40 seconds, at 59.5°C for 35 seconds, and at 71.5°C for 50 seconds. The final extension step was prolonged to 5 minutes. DNA from 50 unrelated individuals was amplified and digested. The 561-bp PCR product (12.5 μ L) was cleaved in appropriate buffer with 15 U of the followed restriction enzymes: *Bam*HI, *Cfo*I, *Eco*RI, *Eco*RV, *Hae*III, *Hind*III, *Mva*I, *Pst*I, *Rsa*I, *Sau*3A, *Taq*I, and *Xho*I (Boehringer Mannheim) at a total volume of 25 μ L at 37°C (65°C for *Taq*I) overnight. The *Hae*III digest revealed a common polymorphism. No polymorphisms were demonstrated with the other restriction enzymes.

Both alleles of the *Hae* III polymorphism were registered in the EMBL database (X74984 and U00699).

Statistical Analysis

The frequencies of the alleles in both groups studied were determined by gene counting, and their associations with biochemical parameters were determined by χ^2 analysis with the Yates correction and with ANOVA.

Results

The frequency of the T allele (restriction site absent) and T/T homozygotes was significantly lower in the control group (35.2% and 15.6%, respectively) than in MI survivors (49.2% and 27.5%, respectively; OR=1.781; 95% CI, 1.286 to 2.465;

$P=0.0005$ for the allele frequency and $P=0.0049$ for the genotype frequency) (Table).

No association between the C(−260)→T polymorphism and total or LDL cholesterol, apolipoprotein B, triglycerides, blood pressure, or body mass index (data not shown) was found in the population sample. No significant differences in conventional risk factors (total, LDL, and HDL cholesterol; body mass index; frequencies of diabetes and hypertension; and smoking) between the patient and control groups were obtained (data not shown).

In healthy volunteers, the density of monocyte CD14 receptors was significantly higher ($P=0.0028$) in the T/T homozygotes ($107\,900 \pm 16\,300$) than in the C/T heterozygotes ($76\,600 \pm 13\,300$) or C/C homozygotes ($76\,000 \pm 13\,700$).

Discussion

Recently, growing evidence has suggested that in addition to the already well-established risk factors (hypercholesterolemia, diabetes, obesity, hypertension, and some others), infection may also be an important risk factor for the development of atherosclerosis and consequently for MI. Among other things,^{12,13} an association was reported between atherosclerosis and infection with Gram-negative bacteria (namely, *Chlamydia pneumoniae*²) and with the presence of their endotoxins (LPSs) in the plasma.¹⁴ LPSs stimulate the synthesis of interleukins and growth factors in monocytes³ and endothelial cells.¹⁵ As a consequence of this increased production, the adhesion of monocytes, leukocytes, and thrombocytes to the vessel wall is also increased.¹⁶

With infection, as with other risk factors for atherosclerosis, one would also suppose that the genetic makeup of individuals would influence the sensitivity to this stimulus. One of the candidate genes is the gene for the CD14 receptor (binds the complex LPS–LPS binding protein formed in the plasma),¹⁷ which plays an important role in the activation of monocytes by LPS. Such activated monocytes can subsequently initiate the process of atherosclerosis.

The described polymorphism in the CD14 gene promoter is located near the Sp1 recognition sequence factor, which is necessary for CD14 expression.⁷ Meisel et al¹⁰ recently described a 30% increased density of CD14 receptors in patients in the acute phase of MI. Our data in healthy volunteers without acute illness showed that the C(−260)→T change affects the level of CD14 gene expression, and thus we suppose that this increased density is permanent and genetically determined.

This C(−260)→T change in the CD14 gene is the first reported polymorphism to suggest that the sensitivity of individuals to infection as an eventual risk factor for atherosclerosis is at least partially genetically determined. This genetic predisposition could explain the accumulation of premature atherosclerosis in certain families without the presence of common risk factors such as hyperlipidemia or hypertension.

We present a report of a polymorphism in the CD14 gene that is associated with increased risk of MI. The

C(−260)→T polymorphism near the Sp1 binding site influences the activity of the CD14 promoter. Whether or not the reported association is causal needs to be established in further studies revealing the pathophysiological mechanism of interactions between monocytes, infectious agents, and the vessel wall in connection with the different genotypes for CD14 receptor.

Acknowledgments

This work was supported by grants 4246-2, 3658-3, and 3635-6 from the Internal Grant Agency of the Czech Ministry of Health, and grant 306/96/K220 from the Grant Agency of the Czech Republic.

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