Membrane-bound CD14 (mCD14) recognizes bacterial compounds like lipopolysaccharide (LPS), lipoteichoic acid (LTA), or peptidoglycan (PGN), while soluble CD14 (sCD14) mediates the response of endothelial and epithelial cells, which do not express CD14, to microbes. In 1999, a polymorphism of the cd14 gene within the promoter, C(−159)→T [also referred to as C(−260)→T], was described (2, 6, 14) and shown to be associated with a higher density of mCD14 (6) and higher sCD14 plasma concentrations (2). This was assumed to be the consequence of increased transcription of the CD14 gene in cases of the C→T substitution, but this hypothesis was so far supported only by the findings of LeVan et al., who showed changes in the binding of the transcription factors Sp1, Sp2, and Sp3 in the CD14 promoter (11). Furthermore, the T allele variant of CD14 was identified as a risk factor for myocardial infarction (6, 14). Using a standardized whole-blood assay (4), we investigated whether blood leukocytes of volunteers with the TT variant show stronger inflammatory responses than leukocytes from carriers of the CC genotype when stimulated with CD14-dependent stimuli.

Heparinized blood from 160 healthy volunteers from the University of Konstanz, Germany (84 male, 76 female; mean age, 30; range, 20 to 70), was incubated with or without 1 μg/ml LPS from Salmonella enterica serovar Abortus-equi (Sigma). The release of interleukin-1β (IL-1β), IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α), gamma interferon (IFN-γ), and granulocyte colony-stimulating factor (G-CSF) was measured by enzyme-linked immunosorbent assay (ELISA), and the serum levels of TNF-α and of the proinflammatory marker “C-reactive protein” were determined by high-sensitivity ELISA (15). Genotyping of DNA (extracted from blood by using a QIAamp DNA blood mini kit [QIAGEN]) for the CD14 polymorphism (14), revealed an incidence of a 24% CC, 51% CT, and 25% TT genotype. Analysis of basal and LPS-induced cytokines dependent on genotype did not result in a significant difference between

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Critical Investigation of the CD14 Promoter Polymorphism: Lack of a Role for In Vitro Cytokine Response and Membrane CD14 Expression

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Blood of volunteers, genotyped for the CD14 C(−159)→T polymorphism, showed no difference in cytokine release when stimulated with nine CD14-dependent immune stimuli. An analysis of the published data on the proposed association of CD14 genotype with membrane CD14 density revealed no significant correlation, questioning a functional impact of the CD14 polymorphism.
0.20 for TT). This also held true for stimulation with the CD14-independent stimulus phorbol myristate acetate.

So far, only a few studies have investigated LPS-inducible TNF-α release of the different CD14 genotypes using monocytes (5), peripheral blood mononuclear cells (10) or blood (3). Kondo et al. reported increased LPS-induced TNF-α release for the TT genotype (10), and Eng et al. claimed a tendency toward higher TNF-α production in the TT group when stimulated with LPS and a significant difference in response to C. pneumoniae (3). In contrast, Heesen et al. observed no difference between the three genotypes (5), which is in line with our findings. It is important to note that in the studies by Kondo et al. (10) and Eng et al. (3), the standard deviations are large, and in the former report, the TT group already had sevenfold-higher basal TNF-α levels than did the CC or CT group (10).

Our finding that monocytes from volunteers with the CC genotype were as sensitive as from donors with the TT genotype, with presumably higher mCD14 expression, was surprising. We therefore redressed the current knowledge on the phenotype of this polymorphism. The initial report on the CD14 polymorphism found a higher density of mCD14 among volunteers with the TT genotype (6). This was confirmed by only one group (3), while two other groups found no association between genotype and mCD14 expression (5, 8). As a simple evaluation, we normalized the data from each of the four studies to the mean mCD14 density of the CC group in the respective study: compared to the 70 CC subjects, the 67 TT subjects showed on average 10% higher mCD14 (109.9%). Statistical significance could not be tested since all studies used different or no ways of expressing variance. A power analysis assuming homogeneity of variances indicates that with the given coefficient of variation (CV) of 25%, a significance level of 5% and a power of 80%, this difference would become significant only at group sizes larger than 100 (S.-Plus 6.2; MathSoft). The association of the TT genotype with myocardial infarction suggested a stronger inflammatory response in subjects with the TT genotype due to increased CD14 levels. However, our analysis does not support an association of the homozygous TT genotype with a relevant increase in mCD14 expression or consequently increased sensitivity to inflammatory stimuli. It should also be noted that the number of studies which do not find a correlation of the CD14 TT genotype with the progression of coronary artery disease (1, 7–9, 12, 13, 16) is increasing.

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REFERENCES