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The CD14-260 C => T promoter polymorphism co-segregates with the tumor necrosis factor-alpha (TNF-alpha)-308 G => A polymorphism and is associated with the interleukin-1beta (IL-1beta) synthesis capacity of human leukocytes

Abstract

Genetic variations contribute to the interindividual variance in the cytokine response to endotoxin. The gene of tumor necrosis factor-alpha (TNF-alpha) carries a polymorphism at position -308 of the promoter, consisting of a G/A exchange. To further elucidate the inherited mechanisms influencing cytokine levels, healthy human blood donors were studied. Genotyping for the TNF-alpha -308 and the CD14 -260 C/T promoter polymorphisms was carried out by real-time polymerase chain reaction assay using specific fluorescence-labelled hybridisation probes. A human whole blood assay was used to study the leukocyte TNF-alpha and IL-1beta synthesis capacity upon endotoxin stimulation. We found a linkage disequilibrium between the TNF-alpha -308 G/A and the CD14 -260 C/T polymorphisms ($p = 0.043$). The CD14 -260 polymorphism was associated with IL-1beta levels ($p = 0.033$) and higher values were found in C homozygotes. No association was found between the CD14 -260 genotypes or the TNF-alpha -308 - CD14 -260 genotypes and the TNF-alpha response.

Full text

INTRODUCTION

A major trigger for the release of cytokines is the binding of lipopolysaccharide (LPS) to the monocyte surface receptor CD14. Consequently, monocytes produce and release cytokines such as tumor necrosis factor-alpha (TNF-alpha) and IL-1beta. Recent research on the interindividual variance in cytokine release elucidated the role of genetic polymorphisms [1]. The genes for CD14 [2] and TNF-alpha [3] carry polymorphisms within the promoter region regulating gene transcription.

The TNF-alpha polymorphism is based on a G/A exchange at position -308 of the promoter region [3]. Two *in vitro* studies demonstrated that the A allele is associated with higher TNF-alpha production [3, 4], a result contrasted by the findings of two other groups [5, 6]. In their report on healthy human blood donors, Louis *et al.* [7] observed an association between this -308 polymorphism and the *ex vivo* TNF-alpha response to LPS. Subjects carrying an A allele had significantly higher TNF-alpha concentrations than G homozygotes. Mira and colleagues [8] reported a higher frequency of the A allele in patients with septic shock compared to a control group. Another group found a higher mortality rate from septic shock in carriers of an A allele [9]. These reports provided

convincing evidence for a role of this polymorphism in the clinical course of septic shock.

Baldini *et al.* [2] discovered the CD14 genetic polymorphism at position -260. This polymorphism consists of a C/T single nucleotide exchange. In children, the plasma levels of soluble CD14 were significantly higher in T homozygotes compared with individuals homozygous for the C allele [2]. Two large clinical studies found this polymorphism associated with the risk for myocardial infarction [10, 11]. Individuals homozygous for the T nucleotide at position -260 had an increased risk for myocardial infarction, with an even higher association in patients without other risk factors such as hypertension or smoking [10]. In healthy blood donors, Hubacek *et al.* [10] found a higher monocyte CD14 density in TT carriers, a result that was challenged by our group [12]. We were unable to find an association between this polymorphism and the monocyte CD14 density, the plasma concentrations of soluble CD14 or the TNF-alpha concentrations after endotoxin stimulation of human whole blood.

In the present study, we found a linkage disequilibrium between the TNF-alpha -308 and the CD14 -260 promoter polymorphisms. Moreover, we studied the association between the genotypes and the TNF-alpha and IL-1beta response to stimulation of the LPS receptor CD14 in a whole blood assay.

METHODS

After approval by the local ethics committee and written informed consent, healthy Caucasian blood donors were studied. Venous blood samples were taken.

Genotyping for the CD14 -260 and the TNF-alpha -308 polymorphisms

Genomic DNA was isolated from the venous blood sample according to standard protocols. Twenty-80 ng genomic DNA (1 muL) were used. Real-time polymerase chain reaction (PCR) assays with specific fluorescence-labelled hybridisation probes were used for genotyping [13, 14]. The 10 muL PCR mixture contained 1 muL reaction buffer (LightCycler DNA master hybridization probes 10C buffer▒, 1.75 mmol/l, Roche Diagnostics, Basel, Switzerland). For the CD14 -260 genotyping, 0.5 mumol/l of the primers (sense: 5'-GGTGCCAACAGATGAGGTTCAC, antisense: 5'-CTTCGGCT-GCCTCTGACAGTT)

as well as 0.2 mumol/l of the detection probe specific for the T allele (5'-LC Red640-TTCCTGTTACGGCCCCCCT-p; the 3'-end was phosphorylated to block extension), and the anchor probe (5'-GGAGACACAGAACCCTAGATGCCCTGCA-fluoresceine) were used. The PCR conditions were: initial denaturation at 95° C for 120 s, followed by 60 cycles of denaturation (95° C for 0 s, 20° C/s), annealing (55° C for 10 s), and extension (72° C for 10 s). The melting curve consisted of 1 cycle at 95° C for 0 s, 45° C for 10 s, and then increasing the temperature to 95° C at a slope of 0.2° C/s.

For the TNF -308 genotyping 0.5 mumol/l of the primers (sense: 5'-AAGGAAACAGACCACAGACCTG, antisense: 5'-GGTCTTCTGGGCCACTGAC) as well as 0.2 mumol/l of the detection probe specific for the G allele (5'-AACCCCGTCCCCATGCC) and the anchor probe (5'-CAAAACCTATTGCCTCCATTTCTTTTGGGGAC) were used. Sixty PCR cycles were run with one PCR cycle consisting of denaturation (95° C for 0 s, 20° C/s), annealing at 57° C for 15 s, and extension (72° C for 10 s).

The thermocycler was a LightCycler instrument (Roche Diagnostics).

Ex vivo *lipopolysaccharide stimulation of whole blood TNF-alpha and IL-1beta release*.

The TNF-alpha and IL-1beta responses of human whole blood to lipopolysaccharide (LPS)-stimulation were assessed as previously described [15]. Heparin-anticoagulated venous blood was diluted 1:1 (v/v) with RPMI 1640 (Gibco BRL, Karlsruhe, Germany). One hundred ng/ml endotoxin (*E. coli* O2:B22) were added. After 4 hours of incubation at 37° C, the samples were centrifuged and the supernatants were stored at - 20° C. The concentrations of TNF-alpha and IL-1beta were determined by measuring immunoreactivity using of chemiluminescence (Immulite, DPC Biermann, Bad Nauheim, Germany).

Statistical analysis

Statistical analysis was performed with the SPSS for Windows Release 10.0.7 program. Values are given as median (minimum-maximum). Non-parametric tests (Wilcoxon Mann Whitney or Kruskal-Wallis test) were used to compare the cytokine concentrations between the genotypes. The association between the

TNF-alpha -308 and the CD14 -260 promoter polymorphisms was assessed by Cramer's V test. P values < 0.05 were considered as statistically significant.

RESULTS

Complete TNF-alpha -308 and CD14 -260 genotyping, as well as TNF-alpha data were gathered for 132 individuals. Forty six subjects had the TNF-alpha -308 genotype AG, 86 were G homozygous. The CD14 -260 genotype frequencies were: 38 CC, 48 CT, and 46 T homozygous. There was a strong linkage disequilibrium between the two polymorphisms (p = 0.043), 42% of carriers of the TNF-alpha -308 genotype GG were homozygous for the CD14 T allele. Table 1 gives the genotype distribution.

Carriers of the TNF-alpha -308 genotype GA produced significantly higher TNF-alpha levels in response to endotoxin than G homozygotes (4,295 (175-7,610) *versus* 2,490 (401-6,990) pg/ml, p = 0.04). The CD14 -260 genotypes TT, CT, and CC did not differ in their ability to produce TNF-alpha (Table 2). However, there was a significant difference in the IL-1beta levels after endotoxin stimulation between the three CD14 -260 genotypes (p = 0.033) with significantly higher values in TT carriers compared to C homozygotes.

The TNF-alpha levels of the TNF-alpha -308 - CD14 -260 genotypes are given in Table 3. There was no difference in the TNF-alpha concentrations between all genotypes.

DISCUSSION

To our knowledge, this is the first report which shows a linkage disequilibrium between the TNF-alpha -308 G/A and the CD14 -260 C/T promoter polymorphisms. TNF-alpha -308 heterozygotes produced significantly higher TNF-alpha levels than G homozygotes in the human whole blood endotoxin stimulation assay. This result is in agreement with previous *in vitro* reporter gene assays [3, 4], as well as an *ex vivo* stimulation study [7]. Westendorpp *et al.* [1] found that 60% of the TNF-alpha response is determined genetically. However, so far there are conflicting results on the known TNF-alpha polymorphisms. *In vitro* and *ex vivo* studies demonstrated that the TNF-alpha -308 A allele is associated with a higher TNF production [3, 4, 7]. Warzocha *et al.* [16] were able to show an impact of this polymorphism on the outcome of non-Hodgkin's

lymphoma. An association with the TNF-alpha levels in tear fluid of patients with scarring trachoma was also described [17]. These findings are in contrast with results obtained by others who did not find an association between the TNF-alpha -308 promoter polymorphism and TNF-alpha RNA in reporter gene assays [5, 6]. The results of the *ex vivo* whole blood stimulation assay used in our study also revealed a role of the promoter polymorphism for TNF-alpha synthesis. The TNF-alpha and the CD14 genes are located on human chromosome 6 [18] and 5 [19], respectively. We found a linkage disequilibrium between these two polymorphisms with 40% of the TNF-alpha -308 G homozygotes being CD14 -260 T homozygous. From our data, the functional implication of this linkage remains unclear. Moreover, the mechanism leading to a linkage between genes located on two different chromosomes remains to be elucidated.

We determined TNF-alpha and IL-1beta levels after endotoxin stimulation and analyzed the association with the TNF-alpha -308 and CD14 -260 genotypes. The lack of association between the CD14 -260 genotypes and the TNF-alpha response, as found in our study, is in line with a previous report [12]. Moreover, we could not show a significant difference in the TNF-alpha response between the TNF-alpha -308 -CD14 -260 genotypes. As a major result of our study, we found an association between the CD14 -260 polymorphism and the IL-1beta response to endotoxin. Carriers of the genotype CC had higher IL-1beta levels than T homozygotes. So far, two polymorphisms in the promoter region of the IL-11beta gene, at positions -31 and -511, have been described which are in near-complete linkage [20]. The IL-1beta -31 involving a TATA sequence affected DNA binding in electrophoretic mobility-shift assays. The IL-1beta -511 polymorphism was found to mediate its effect by linkage disequilibrium with the TATA box polymorphism. Our results suggesting an association between the CD14 -260 polymorphism and IL-1beta levels, could theoretically be related to differences in the CD14 receptor density in the various CD14 -260 genotypes. Hubacek and colleagues [10] reported a higher CD14 density on circulating monocytes of T homozygotes. In a previous study, we were unable to show any association between this polymorphism and the CD14 density on human monocytes or soluble CD14 levels [12]. It is therefore daring to speculate that the CD14 polymorphism is only a marker of another genetic factor which is the true mediator of IL-1beta concentrations.

CONCLUSION

In summary, this study demonstrates a linkage disequilibrium between the TNF-alpha -308 and the CD14
-260 genotypes. The CD14 -260 genotypes is associated with the IL-1beta response to endotoxin, whereas no association was seen between CD14 -260 or TNF-alpha-308 -CD14 -260 genotypes and the TNF-alpha levels.

References

1. Westendorp R G, Langermans J A, Huizinga T W, Elouali A H, Verweij C L, Boomsma D I, Vanden-Broucke J P. 1997. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 349: 170.

2. Baldini M, Lohman I C, Halonen M, Erickson R P, Holt P G, Martinez F D. 1999. A polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am. J. Respir. Cell Mol. Biol.* 20: 976.

3. Wilson A G, Symons J A, McDowell T L, McDevitt H O, Duff G W. 1997. Effects of a polymorphism in the human tumor necrosis factor-alpha promoter on transcriptional activation. *Proc. Natl. Acad. Sci. USA* 94: 3195.

4. Kroeger K M, Carville K S, Abraham L J. 1997. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol. Immunol.* 34: 391.

5. Brinkman B M, Zuijdeest D, Kaijzel E L, Breedveld F C, Verweij C L. 1995-96. Relevance of the tumor necrosis factor-alpha (TNF-alpha) -308 promoter polymorphism in TNF-alpha gene regulation. *J. Inflamm.* 46: 32.

6. Stuber F, Udalova I A, Book M, Drutskaya L N, Kuprash D V, Turetskaya R L, Schade F U, Nedospasov S A. 1995-1996. -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. *J. Inflamm.* 46: 42.

7. Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M, De Groote D, Louis R, Belaiche J. 1998. Tumor necrosis factor (TNF) gene polymorphism influences TNF-alpha production in

lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin. Exp. Immunol.* 113: 401.

8. Mira J P, Cariou A, Grall F, Delclaux C, Losser M R, Heshmati F, Cheval C, Monchi M, Teboul J L, Riche F, Leleu G, Arbibe L, Mignon A, Delpech M, Dhainaut J F.1999. Association of TNF2, a TNF promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. *JAMA* 282: 561.

9. Tang G J, Huang S L, Yien H W, Chen W S, Chi C W, Wu C W, Lui W Y, Chiu J H, Lee T Y. 2000. Tumor necrosis factor gene polymorphism and septic shock in surgical infection. *Crit. Care Med.* 28: 2733.

10. Hubacek J A, Rothe G, Pit'ha J, Skodova Z, Stanek V, Poledne R, Schmitz G. 1999. C(-260) => T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 99: 3218.

11. Unkelbach K, Gardemann A, Kostrzewa M, Philipp M, Tillmanns H, Haberbosch W. 1999. A new promoter polymorphism in the gene of lipopolysaccharide receptor CD14 is associated with expired myocardial infarction in patients with low atherosclerotic risk profile. *Arterioscler. Thromb. Vasc. Biol.* 19: 932.

12. Heesen M, Bloëmeke B, Schluter B, Heussen N, Rossaint R, Kunz D. 2001. Lack of association between the -260 C => T promoter polymorphism of the endotoxin receptor CD14 gene and the CD14 density of unstimulated human monocytes and soluble CD14 plasma levels. *Intensive Care Med.* 27: 1770.

13. Heesen M, Kunz D, Rossaint R, Blömeke B. 2000. Real-time PCR assay with fluorescent hybridization probes for the rapid genotyping of the CD14 promoter polymorphism. *Clin. Chem.* 46: 1866.

14. Moos V, Rudwaleit M, Herzog V, Hohlig K, Sieper J, Muller B. 2000. Association of genotypes affecting the expression of interleukin-1beta or interleukin-1 receptor antagonist with osteoarthritis. *Arthritis Rheum.* 43: 2417.

15. Flach R, Majetschak M, Heukamp T, Jennissen V, Flohe S, Borgermann J, Obertacke U, Schade F U. 1999. Relation of *ex vivo* stimulated blood cytokine synthesis to post-traumatic sepsis. *Cytokine* 11: 173.

16. Warzocha K, Ribeiro P, Bienvenu J, Roy P, Charlot C, Rigal D, Coiffier B, Salles G. 1998. Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. *Blood* 91: 3574.

17. Conway D J, Holland M J, Bailey R L, Campbell A E, Mahdi O S, Jennings R, Mbena E, Mabey D C. 1997. Scarring trachoma is associated with polymorphism in the tumor necrosis factor-alpha (TNA-alpha) gene promoter and with elevated TNF-alpha levels in tear fluid. *Infect. Immun.* 65: 1003.

18. Nedwin G E, Naylor S L, Sakaguchi A Y, Smith D, Jarrett-Nedwin J, Pennica D, Goeddel D V, Gray P W. 1985. Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. *Nucleic Acids Res.* 13: 6361.

19. Goyert S M. Ferrero E, Rettig W J, Yenamandra A K, Obata F, Le Beau M M. 1988. The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. *Science* 239: 497.

20. El-Omar E M, Carrington M, Chow W H, McColl K E, Bream J H, Young H A, Herrera J, Lissowska J, Yuan C C, Rothman N, Lanyon G, Martin M, Fraument J F Jr, Rabkin C S. 2000. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 404: 398.

Figures

Table 1
Frequencies of the TNF-α -308 – CD14 -260 genotypes

	CD14 -260 CC	CT	TT	
AG	10 (22%)	26 (56%)	10 (22%)	n = 46
		TNF-α -308		
GG	28 (33%) n = 38	22 (25%) n = 48	36 (42%) n = 46	n = 86

Table 2
TNF-α and IL-1β responses (pg/ml) of whole blood to endotoxin stimulation, according to CD14 -260 genotypes

		CD14 -260 genotypes		
		TT	CT	CC
TNF-α	Median	2,293	2,925	2,790
	Minimum	401	1,019	175
	Maximum	5,759	6,990	7,610
IL-1β	Median	329*	662	647
	Minimum	76	119	163
	Maximum	875	2,335	2,150

*: **p** = 0.014 *versus* CD14 -260 CC.

Table 3
TNF-α levels (pg/ml) after whole blood stimulation with endotoxin, according to TNF-α -308 – CD14 -260 genotypes

	TNF-α levels (pg/ml)		
TNF-α -308 - CD14 -260 Genotype	Median	Minimum	Maximum
GA-CC	4,295	175	7,610
GA-CT	3,102	1,770	6,460
GA-TT	5,196	1,964	5,803
GG-CC	2,610	1,230	6,245
GG-CT	3,430	1,721	6,990
GG-TT	2,054	401	5,560

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