Physiol. Res. 48: 323-326, 1999

SHORT COMMUNICATION

The Common cDNA and Amino Acid Sequences of the CD14 (Myeloid Cell-Specific Leucine-Rich Glycoprotein) Receptor

J. A. HUBÁČEK, R. POLEDNE

Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Received June 11, 1998
Accepted February 24, 1999

Summary

The CD14 receptor is a myeloid cell specific receptor, which plays a role in the recognition of lipopolysaccharides (endotoxins of Gram-negative bacteria) and cell stimulation. To date, several sequences of the cDNA of the CD14 receptor have been described. We sought to establish whether the substitutions C(230) → G, and G(560) → A are polymorphic or if they result from a PCR or sequencing error. Using two mismatched PCRs, we confirmed (on 75 unrelated probands) that the substitutions are not due to common polymorphisms. The common cDNA sequence has the C in position 230 and G in position 560. This corresponds to the amino acids Ala and Cys in positions 77 and 187, respectively.

Key words

CD14 receptor • Monocyte • cDNA • Mismatched PCR

Monocytes and their activation play an important role in the first phase of atherogenesis. The monocytes could be activated by endotoxins (lipopolysaccharides - LPS) from the envelope of Gram-negative bacteria.

The CD14 receptor (myeloid cell-specific leucine-rich glycoprotein) is localized on the surface of all mature myeloid cells (largely on monocytes) and occurs in soluble form in the plasma. The role of the CD14 receptors is to enhance cell sensitivity to LPS. LPS recognition by cells is the one of the first steps of the defense cascade. The effects of LPS on cells (enhanced production of cytokines, growth and coagulations factors, prostaglandins, etc.) have been experimentally demonstrated (for review see Schumann *et al.* 1994). The CD14 receptor thus plays an important role in the monocyte activation, and could therefore play a role in the development of atherosclerosis.

The CD14 receptor gene is localized on human chromosome 5 and consists of about 3900 nucleotides, which are organized in two exons and one intron. The promoter of the CD14 gene has been sequenced and characterized (Zhang *et al.* 1994).

To date, sequence of the cDNA CD14 receptor has been described several times (Ferrero and Goyzert 1988, Simmons *et al.* 1989, Setoguchi *et al.* 1989, EMBL database X13334, X06882, M86511). When comparing these sequences, two differences are detectable. The first is the C → G substitution at position 230 (Ala77 → Gly),

Fax +420 2 293 894
E-mail physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

the second one concerns the G → A substitution in position 560 (Cys187 → Tyr) of the cDNA sequence (position 1 as the first base of the start codon).

The CD14 sequences for human, mouse, rat and rabbit reveal a considerable conservation between species, with interspecies protein sequence homology between 60% and 80% (Takai *et al.* 1997). Both putative polymorphisms studied are localized in these conserved regions. Changes in these regions could influence the structure of the protein and, therefore, the function of the CD14 receptor.

```
1    ATGGAGCGCGCGTCCTGCTTGTTGCTGCTGCTGCTGCCGCTGGTGCACGTCTCTGCGACC   60
     M  E  R  A  S  C  L  L  L  L  L  L  P  L  V  H  V  S  A  T  -

61   ACGCCAGAACCTTGTGAGCTGGACGATGAAGATTTCCGCTGCGTCTGCAACTTCTCCGAA  120
     T  P  E  P  C  E  L  D  D  E  D  F  R  C  V  C  N  F  S  E  -

                          aagccttccagtgtgtgtctgcag
121  CCTCAGCCCGACTGGTCCGAAGCCTTCCAGTGTGTGTCTGCAGTAGAGGTGGAGATCCAT  180
     P  Q  P  D  W  S  E  A  F  Q  C  V  S  A  V  E  V  E  I  H  -

                                                    gcGgggcgcc
181  GCCGGCGGTCTCAACCTAGAGCCGTTTCTAAAGCGCGTCGATGCGGACGCCGACCCGCGG  240
     A  G  G  L  N  L  E  P  F  L  K  R  V  D  A  D  A  D  P  R  -

     gtcatacgactgtg
241  CAGTATGCTGACACGGTCAAGGCTCTCCGCGTGCGGCGGCTCACAGTGGGAGCCGCACAG  300
     Q  Y  A  D  T  V  K  A  L  R  V  R  R  L  T  V  G  A  A  Q  -

301  GTTCCTGCTCAGCTACTGGTAGGCGCCCTGCGTGTGCTAGCGTACTCCCGCCTCAAGGAA  360
     V  P  A  Q  L  L  V  G  A  L  R  V  L  A  Y  S  R  L  K  E  -

361  CTGACGCTCGAGGACCTAAAGATAACCGGCACCATGCCTCCGCTGCCTCTGGAAGCCACA  420
     L  T  L  E  D  L  K  I  T  G  T  M  P  P  L  P  L  E  A  T  -

421  GGACTTGCACTTTCCAGCTTGCGCCTACGCAACGTGTCGTGGGCGACAGGGCGTTCTTGG  480
     G  L  A  L  S  S  L  R  L  R  N  V  S  W  A  T  G  R  S  W  -

      tcgccgagctgcagcagtggctcaag
481  CTCGCCGAGCTGCAGCAGTGGCTCAAGCCAGGCCTCAAGGTACTGAGCATTGCCCAAGCA  540
     L  A  E  L  Q  Q  W  L  K  P  G  L  K  V  L  S  I  A  Q  A  -

                              ccttgtccaagcgcggaagggccggga
541  CACTCGCCTGCCTTTTCCTGCGAACAGGTTCGCGCCTTCCCGGCCCTTACCAGCCTAGAC  600
     H  S  P  A  F  S  C  E  Q  V  R  A  F  P  A  L  T  S  L  D  -

601  CTGTCTGACAATCCTGGACTGGGCGAACGCGGACTGATGGCGGCTCTCTGTCCCCACAAG  660
     L  S  D  N  P  G  L  G  E  R  G  L  M  A  A  L  C  P  H  K  -

661  TTCCCGGCCATCCAGAATCTAGCGCTGCGCAACACAGGAATGGAGACGCCCACAGGCGTG  720
     F  P  A  I  Q  N  L  A  L  R  N  T  G  M  E  T  P  T  G  V  -

721  TGCGCCGCACTGGCGGCGGCAGGTGTGCAGCCCCACAGCCTAGACCTCAGCCACAACTCG  780
     C  A  A  L  A  A  A  G  V  Q  P  H  S  L  D  L  S  H  N  S  -

781  CTGCGCGCCACCGTAAACCCTAGCGCTCCGAGATGCATGTGGTCCAGCGCCCTGAACTCC  840
     L  R  A  T  V  N  P  S  A  P  R  C  M  W  S  S  A  L  N  S  -

841  CTCAATCTGTCGTTCGCTGGGCTGGAACAGGTGCCTAAAGGACTGCCAGCCAAGCTCAGA  900
     L  N  L  S  F  A  G  L  E  Q  V  P  K  G  L  P  A  K  L  R  -

901  GTGCTCGATCTCAGCTGCAACAGACTGAACAGGGCGCCGCAGCCTGACGAGCTGCCCGAG  960
     V  L  D  L  S  C  N  R  L  N  R  A  P  Q  P  D  E  L  P  E  -

961  GTGGATAACCTGACACTGGACGGGAATCCCTTCCTGGTCCCTGGAACTGCCCTCCCCCAC 1020
     V  D  N  L  T  L  D  G  N  P  F  L  V  P  G  T  A  L  P  H  -

1021 GAGGGCTCAATGAACTCCGGCGTGGTCCCAGCCTGTGCACGTTCGACCCTGTCGGTGGGG 1080
     E  G  S  M  N  S  G  V  V  P  A  C  A  R  S  T  L  S  V  G  -

1081 GTGTCGGGAACCCTGGTGCTGCTCCAAGGGGCCCGGGGCTTTGCCTAA             1128
     V  S  G  T  L  V  L  L  Q  G  A  R  G  F  A  *
```

Fig. 1. *The common cDNA and protein sequences of the human CD14 receptor. The localization of the primers are given. The putative polymorphic nucleotides are underlined. The mismatched bases in primers are in bold italics.*

To determine whether the substitutions are common polymorphisms or if they are artifacts resulting from a polymerase chain reaction (PCR) or a sequencing mistake, we established two PCR´s with mismatched primers. A mismatched primer contains a mismatch at 3´ end which introduces a new restriction site in the product only when the appropriate nucleotide in the polymorphic allele is amplified. Thus, a putative polymorphic nucleotide exchange, which is not localized at the naturally present restriction site, could be recognized.

Table 1. The primer sequence and appropriate restriction enzymes for detection of the putative polymorphisms in human CD14 receptor gene.

	Primer sequences (5' → 3')	Restriction enzyme	PCR product size (bp)	Expected restriction fragments size (bp)
GA-1	aag cct tcc agt gtg tgt ctg cag	Sty I	107	81 + 26
GA-2	gtg tca gca tac tgc cgc ggg gcg			
CG-1	tcg ccg agc tgc agc agt ggc tca ag	Nar I	115	91 + 24
CG-2	agg gcc ggg aag gcg cga acc tgt tcc			

PCR was performed in the total volume of 50 µl (100–300 ng genomic DNA, 1 U Taq DNA polymerase, 50 pmol of each primer, 200 nmol of each dNTP, 1.5 mmol Mg^{2+}) on a Perkin Elmer 9600 thermocycler. All chemicals were provided by Boehringer (Mannheim, Germany). For the G → A substitution, the initial denaturation was 95 °C for 1.5 min followed by 35 cycles at 93 °C for 10 s, 58 °C for 30 s, 72 °C for 30 s. For the C → G substitution, the initial denaturation was 95 °C for 1.5 min followed by 35 cycles at 93 °C for 10 s, 71.5 °C for 30 s (two steps PCR). The primer sequences, sizes of PCR products and expected restriction fragments with the corresponding restriction enzymes are summarized in Table 1. Fifteen microliters of the PCR products were digested with 10 U of the appropriate restriction enzyme in a total volume of 25 µl overnight at 37 °C. The fragments were separated on 3 % agarose gel in 0.5x TBE buffer and visualized with ethidium bromide.

For both putative polymorphisms, 75 randomly selected unrelated probands have been analyzed. No differences in the restriction maps of the restricted PCR products were detected. If the amino acid exchanges are polymorphic, the frequencies are under 1 % in the population and should therefore be described as a rare mutation. It could not be excluded that the studied discrepancies represented PCR or sequencing errors caused by inaccuracy of enzymes used for these reactions. We have confirmed, that the common CD14 cDNA sequence has the C in position 230 and G in position 560 (Fig. 1). This corresponds to the interspecies conserved amino acids Ala in the position 77 and Cys in the position 187, respectively.

Acknowledgments

This work was supported by grant No. 4246-2/97 awarded by the Internal Grant Agency of the Czech Ministry of Health. The excellent technical assistance of M. Šimáčková is gratefully acknowledged.

References

FERRERO E, GOYERT SM: Nucleotide sequence of the gene encoding the monocyte differentiation antigen, CD14. *Nucleic Acids Res* **16**: 4173, 1988.

SCHUMANN RR, RIETSCHEL ET, LOPPNOW H: The role of CD14 and lipopolysaccharide-binding protein (LBP) in the activation of different cell types by endotoxin. *Med Microbiol Immunol* **183**: 279-297, 1994.

SETOGUCHI M, NASU N, YOSHIDA S, HIGUSHI Y, AKIZUKI S, YAMAMOTO S: Mouse and human CD14 (myeloid cell-specific leucine-rich glycoprotein) primary structure deduced from cDNA clones. *Biochim Biophys Acta* **1008**: 213-222, 1989.

SIMMONS DL, TAN S, TENEN DG, NICHOLSON-WELLER A, SEED B: Monocyte antigen CD14 is a phospholipid anchored membrane protein. *Blood* **73**: 284-289, 1989.

TAKAI N, KATAOKA M, HIGUSCHI Y, MATSUURA K, YAMAMOTO S: Primary structure of rat CD14 and characteristics of rat CD14, cytokine and NO synthase mRNA expression in mononuclear phagocyte system cells in response to LPS. *J Leukocyte Biol* **61**: 736-744, 1997.

ZHANG DE, HETHERINGTON CJ, TAN S, DZIENNIS SE, GONZALES DA, CHEN HM, TENEN DG: Sp1 is a critical factor for the monocytic specific expression of human CD14. *J Biol Chem* **269**: 11425-11434, 1994.

Reprint requests

J. A. Hubáček, Institute for Clinical and Experimental Medicine, Laboratory of Atherosclerosis Research, Vídenská 1958/9 140 21 Prague 4, Czech Republic. Fax: + 420 2 472 15 74, E-mail: jaroslav.hubacek@medicon.cz