Molecular Cloning of Bovine CD14 gene

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ABSTRACT. Genomic DNA encoding bovine CD14 was isolated from a bovine (Holstein) genomic library. Utilizing PCR fragment of mouse CD14 gene as a probe, we screened 9×10^5 plaques and obtained 3 clones containing the bovine CD14 gene. DNA sequencing showed that bovine CD14 gene encodes 373 amino acids, and the coding sequence was separated by a 90 nt intron. The identity of the deduced amino acid sequence of bovine CD14 was 61–73% to those of mouse, rabbit and human. Northern blot analysis revealed that CD14 mRNA (1.5 kb) was expressed in the lung. Expression of CD14 mRNA was stimulated about 2-fold in bovine peripheral blood macrophage activated with LPS *in vitro*.—**KEY WORDS:** bovine, CD14, genomic DNA cloning.

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CD14 is a myeloid membrane glycoprotein which serves as a receptor for complexes of lipopolysaccharide (LPS) and LPS-binding protein (LBP) [13, 16, 19]. Most prominent cellular response for LPS is that monocytes and macrophages extensively produce pro-inflammatory cytokines such as IL-1 α , β and TNF α [1, 12, 18, 19]. The precise mechanisms of LPS-LBP signal transduction via CD14 receptor is yet unclear. Since CD14 is a glycosyl-phosphatidylinositol-anchored protein and lacks the intracellular sequence required for signaling, CD14-associated transducing molecules may be required for LPS signaling [6, 19]. Functional roles of CD14 receptor have been suggested in endotoxemia, sepsis and inflammatory diseases [4, 9, 13, 19]. To investigate the mechanism of LPS-signal-transduction and regulation of CD14 mRNA expression in bovine, we report here the cloning and the nucleotide sequence of bovine CD14 genomic gene.

We amplified a part of mouse CD14 gene (1416–1706 nt position [11]) and used as a probe. PCR was performed in a TAKARA Thermal Cycler PJ-480 for 30 cycles at 94°C for 1 min, 53°C for 1.5 min and 72°C for 1.5 min. The final volume of 100 μ l contained 1 μ g of C57BL/6J mouse genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 200 μ M dNTP, 1 μ M sense primer (5'-AGACCTGTCTGACAATCCTG-3'), 1 μ M anti-sense primer (5'-TTGGCTGGCAGCCCTTTAGG-3') and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer). A PCR product of approximately 300 bp was obtained, and labeled with [α -³²P] dCTP (3000 Ci/mmol, NEN) by random primer labeling kit (Amersham). A bovine genomic DNA library was purchased from CLONTECH (cat. #BL 1015j), and approximately 10⁶ plaques of the library were plated and transferred to nylon filters (Colony/Plaque Screen; NEN). They were hybridized with the [α -³²P] dCTP-labeled probe for 20 hr at 65°C in hybridization mix (1% SDS, 1M NaCl, 10% dextran sulfate, 100 μ g/ml salmon sperm DNA).

After being washed twice with 2XSSC/1% SDS for 15 min at 60°C, the filters were exposed to an Imaging Plate (Fuji Photo Film Co., Ltd.). Three positive clones were obtained, and these three genomic DNAs were digested by XhoI and 7.5 kb fragment were subcloned into XhoI-digested pBluescript SK II+ for sequencing.

DNA sequences were determined by the dideoxy nucleotide chain termination method on a DNA sequencer 373S (Applied Biosystems). The sequences were obtained for both strands. Sequence analysis was performed with Genetyx-Mac software (Software Development Co., Ltd.). Three clones have identical sequence each other within ATG initiation codon to TAA stop codon, one of three clone was sequenced throughly. CD14 genomic DNA sequence is shown in Fig. 1. Splice donor/acceptor sites were confirmed by sequencing of RT-PCR products (data not shown). Coding region was 1119 nt in length, and a 90 nt intron was present just behind an ATG initiation codon. This gene organization is similar to those of other species [2, 3, 8, 11] and its coding sequence was highly homologous to mouse [3, 11, 14], human [2, 15] and rabbit [8] CD14 cDNAs. We could not determine the strict position of the transcription initiation site and polyadenylation signals, mouse CD14 cDNA have about 110 bp of 5' untranslated region and about 80 bp or 220 bp of 3' untranslated region [3, 11].

The deduced amino acid sequence of bovine CD14 was compared and aligned with those of three other mammalian species (Fig. 2). As shown in Table 1, the number of amino acids in bovine CD14 was 373 and the identities between the amino acid sequences of bovine CD14 and the other three mammalian species were 61–73%.

The distribution of the CD14 transcripts was investigated by RT-PCR of poly(A)⁺ RNA from the kidney, lung, brain and liver (Fig. 3). Reverse transcription was carried out with MuLV-RT (Perkin-Elmer) and oligo(dT)₁₆ and PCR was performed in TAKARA Thermal Cycler TP-3000 for 30 cycles at 94°C for 1.5 min, 64°C for 1.5 min and 74°C for 1.5 min. The reaction mixture (100 μ l) contained 500 ng of cDNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 200 μ M dNTP, 0.2 μ M of sense primer (5'-TCCAGCCGACAACCAGAGAGA-3'), 0.2 μ M of anti-sense primer (5'-AAGGCTGGTTGTTGAGATGTC-

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61 agagaggtgagcaactaggccaatgtgtctctgtttcaacacatagacttctctacttttata
121 gaaagctgttacttttaaaattgtggaatcacacattgcacacctgtttacaagctaaactat
181 cctaggagaaaggaacatggcaaccacactccagttctcagctgggaaaatcccaagacaga
241 ggagcctgtgtgagctgcagctccatggggttgcctgaggagtgtggacaggactgcagcagcttc
301 acttttcaacttttcaactttcatgctctgggagagggaaatggcaaccacactccagttggtctt
361 gccttggagaaatcccaaggaaggggggtctgtgtgggtctgcgctctatagggttccacacagag
421 tcagacacagctgtgaggtgactttagtagcagcagcagcagcagacacaaagctggccaat
481 gaagattttccactgtgtctgttcaacttccctgtgtgttccagttggtttaaagactgcactccca
541 ctgcagggtgtgtgacatttcaatttctggtcagggaactaaagtacacacaaagcagcaggg
601 ccaaaaaaaataaataattctgttactttcttaataaaacacatttaaagaaacaaagtcagcaaa
661 aaaaaaaaaaaggagcgtgggggggaagctgttcttttagacaaacttcttctctggaaacta
721 gcccttggaggttgatcaatgaaggttgcagctctctctccacaaactatccagttgaataacc
781 tggagaaaattcaggggaggttgaacttgggttaataaggaagttaataaacacaaacagactga
841 gagaaaagtattggaaaagtaccacagctcgaattcagctgggataggggcaggggagcagtgag
901 aatctctggctgtgaggaaactgggggtatctccttttctccaaacccagacaaagctgggggacg
961 ctgagtcattctgagcactgctcgggtcagggaattgaggggcttgcgaagatcttctctgtg
1021 tatagcccccttccagaaacacatccttcatttgcgaaatttccagggaagagatgcgaactgg
1081 ggtggtgggggaggaagaaaggagggggagcaggggttcaaaaggagggggagcagagcga
1141 catcacaggggttcaataaaactctcagaaggttgcggaaaggagttaaaagacagatagggtttatg
1201 gaggtctggaagccggctgtgagccactgtaaaaggaaagaatccacagtcagcagcagcagcag
1261 agagagagagggcagagggctctgagaatctaccgactattgtgtgagagtatgttcttggggc

M Intron

1321 cgaagcgtgggctattttggggaaaccttaggaacaggcttggggccgcctgacctcgcgtg

1381 tctttccaggtgctgccttccctacctgtctgtctgtcgcgtccgctcactgctgcgtgtg

1441 tctgcgggacacaacagaaacctgcagcttggaacgacgacgatttccggtgtgtctgcaac

1501 ttcacgtatccgaagcctgactctctagcgcgcgttcagtgataggttgcctgcagggtg

1561 gagatcagtgccggcgcgacgacctggaacaggtttctcaaggaggcgcgacaccaaccgg

1621 aagcagtatgctgcacacaaactcaagggtctgcgcgttcggcgactcaagctggcgactgca

1681 caggttctctgctcagcttctgtgtgcgcgttctgcgcgcgtcgggtactctcgttctcaag

1741 gaaactgacgttctgaggacacctggaggtaaacgcgcccaacgcctcgcagcgtctctggaagcc

1801 gctggggcttgcgctcaccacacctcagctctcgttaacgtatcgtggacaacaggaggtgcc

1861 tggctcggcgaaactgcagcagctgggtcagaagcctgggctcagggtgctgaaccttgcccaa

1921 gcacactcgttgccttctcgtgcgcagggtcttccacttccagggcgtccacacctta

1981 gacctgtctgacaaatccagctctcggcgacacaggggctgtagggagctctctgttcggaac

2041 aagttccggccctccaattctagcgttaacgcggggatggagacgcgcgagcggc

2101 gtgtcggcggtcgttcggcgagagggtgcagccccaagactgggacctcagccacacac

2161 tgcgtgcgcgtcagcccgccgggtgctaccgattgtgtgtggcccgagtgcaactaaggtct

2221 ctcaatttctgcttctgtgggtctggagcaagtgcttaagggaactgccccctaaagctcagc

2281 gtgcttgatctcagctgcacaacagctaaggcagcccgacggcgagagctgcagctgccgag

2341 gtaaatgacctgactctggagcggaaatccctttctggaccctggagccctccagcaccaa

2401 aatgacccgatgtctccggcgtggtccagcctgtgcgcgttgccttgacctgacctgggg

2461 gtgtcaggagccctggcgctgtctcaaggagcccgaggcttcgcgttaaggccagggggaag

2521 agaggggaaggaggaatgaattgcctcagattgcctcgggtcctggggagacccctcgcaggac

2581 atctcaaccacacagcctcttacccttcttcttcttcttcttcttcttcttcttcttcttctt

2641 cattgactcagcagatgttttactgggacagctgtctggagcagggaatccattatactttaa

2701 ctttcttctcttaacttcttcttcttcttcttcttcttcttcttcttcttcttcttcttcttctt

2761 ggcagaaaggggtatgaaaaattgagttgtcttgggtcgtgtgttcttctcagggtttct

2821 cctgccatttgggttttcttgaagcagacttactaacctctgtttaaaggagtgttgaagt

2881 tgaacacatgccttggaggaactataatgtttcttcttctgtgtgacttcttcaagagccagc

2941 tcagctgtctctctcccaaggttaataataaacccagaggcatttgccttattcttacc

Fig. 1. Nucleotide and deduced amino acid sequences of bovine CD14 gene. The potential polyadenylation signals are underlined.

Bovine	MVCVPYLLLLLLPSLLRVSA DTTEPCELD DDDFRVCNF TDEPKPDWSSAVQOMVAVEVEI
Human	MERASCLLLLLLP-LVHVSATTEPCELDDEDRCVCNFSEPOPDWSEAFQCVSAVEVEI
Mouse	MERVLG-LLLLL-LVHAS PAPP EPC ELD-DEE-SCSCNFSDPKPDWSSAFNCLGAADVEL
Rabbit	MEVPCLLLLLLP-LLRAS TDTPEPCELD DDDIRVCNF SDEPOPDWSSALQMPAVQVEM
Bovine	SAGGRSLEQFLKGA D--TNPKQYADTIKALRVRRILKLGAAQVPAQLLVAVLRALGYSRLK
Human	HAGGLNLEPFLKRVDADADPROYADTVKALRVRRITVGAAQVPAQLLVGALRVLAYSRILK
Mouse	YGGGRSLEYLLKRVDTEADLGQFTDIKSLKRLTVRAARIPSRILFGALRVLGISGLQ
Rabbit	WGGGHSLEQFLROADLYTDORRYADV KALRVRRITVGAVQVPAQLLVGLRVLGYSRLK
Bovine	EITLEDLEVITG-PTPTPLEAAGPALTTISLRNVSWTTGGAWLGELQOWLKPGLRVINIA
Human	EITLEDLKITGT-MPPLPLEATGLAISLRLNRNVSWATGRSWIAELQOWLKPGLKVLISIA
Mouse	EITLENLEVITGT-APPPLLEATGPDINIINLRNVSWATRDWIAELQOWLKPGLKVLISIA
Rabbit	EIALEDIEVITGTAPPEPPLLEATGPALSTISLRNVSWPKGGAWISELQOWLKPGLQVINIA
Bovine	QAHSLAFPCAGLSTFEALTTLDLSDNPSLGDSGLMAALCPNKFPAQLQYLALRNAGMETPS
Human	QAHSPAFSYEQVRAFPALTSLDLSDNEGLGERGLMAALCPHKFPAIQNLALRNTGMEIPT
Mouse	QAHSLNFSCEQVRVFPALSTLDLSDNPELGERGLISALCPKFPFTLOVLALRNAGMETPS
Rabbit	QAHTLAFSCEQVRTESALTTLDLSDNPELGERGLVAALCPHKFPAQDLALRNAGMKITLO
Bovine	GVCAALAAARVCPQSLDLSHNSLR-VTAPGATRCVWPSPALRSNLNSFAGLEQVPKGLPPK
Human	GVCAALAAAGVCPHSLDLSHNSLRATVNPSAPROMWSSALNSNLNSFAGLEQVPKGLPAK
Mouse	GVCSALAAARVCLQGLDLSHNSLRDA--AGAPSCDWPSQINSLNSLSFTGLKQVPKGLPAK
Rabbit	GVCAALAEAGVCPHLDLSHNSLR---A-DTORCIWPSALNSNLNSLSFTSLQVPKGLPAK
Bovine	LSVLDLSCNKLSPRRDELPEMDNLTLDGNPFLDPGALQHNDPMISGVVPACARSALT
Human	LRVLDLSCNRLNRAPOPELPEMDNLTLDGNPFLVPGTALPHEGSMNSGVVPACARSTLS
Mouse	LSVLDLSYNRLDRNPSPELPEQGNLSLKGNPFL--DSE-SHSEKFNSGVVTAGAPSSQA
Rabbit	LNVLDSLSCNKLNRAPOPELPEKVNLSLDGNPFLVPGASKLOEDLTNSGVFPACPPSPLA
Bovine	MGVSGTIALILOGARGFA
Human	VGVSGTIVLLILOGARGFA
Mouse	VALSGTIALILGDRLEFV
Rabbit	MGMSTGIALILOGARGFI

Fig. 2. Comparison of deduced amino acid sequences of CD14 among four mammalian species. The sequences are from mouse [3, 11, 14], human [2, 15] and rabbit [8] sources. The boxed amino acids are conserved among four species.

Table 1. Amino acid sequence homology of Bovine CD14 to other mammalian species

	Homology (nuc)	Homology (aa)	Length (aa)
Bovine	—	—	373
Mouse	70.4%	61.7%	366
Rabbit	77.0%	72.3%	372
Human	78.6%	73.1%	375

3') and 2.5 units of AmpliTaq Gold polymerase (Perkin-Elmer). PCR products of approximately 1,250 bp were obtained (Fig. 3), and sequenced by DNA sequencer 373S. The sequence of the four fragments were identical to the coding sequence of genomic DNA clone.

A 1.5 kb and 3.5 kb RNA species were detected by northern blot hybridization in bovine lung poly(A)⁺ RNA (Fig. 4a). This results is consistent with a recent report on northern blot of bovine alveolar macrophage CD14 mRNA

by using human CD14 cDNA as a probe [17]. Although we do not determine the transcription initiation site of bovine CD14 mRNA, 1.5 kb RNA is the expected size for bovine CD14 mRNA in comparison with CD14 mRNA of other species [3, 11]. Significance of 3.5 kb RNA band is not known.

It has been shown that CD14 mRNA expression in monocytes and macrophages is upregulated by LPS [7, 10]. We prepared bovine peripheral monocytes from mononuclear cell fraction by an adherent method [5]. Monocytes were cultured with or without LPS (50 µg/ml) for 4 hr, then poly(A)⁺ RNA was extracted and subjected to northern blot analysis. As shown in Fig. 4b, the 1.5 kb RNA expression was enhanced about 2-fold by LPS. The expression of 3.5 kb RNA was also enhanced by LPS. The probe is specific and hybridization/wash condition is stringent, so 3.5 kb RNA may be a precursor of bovine CD14 mRNA. But its character is still to be dissolved.

Further investigation of regulation of CD14 mRNA

Fig. 3. RT-PCR analysis of CD14 transcripts in several tissues. RT-PCR products were electrophoresed on 1.2% agarose (TAE buffer) gel. Odd numbered lanes indicate RT-PCR products (approx.=1,250 bp) and even numbered lanes indicate the negative control (without RT). Lanes 1 and 2, kidney; lanes 3 and 4, lung; lanes 5 and 6, brain; lanes 7 and 8, liver.

Fig. 4. Northern blots of poly(A)⁺ RNAs from bovine tissues using bovine CD14 probe (1718 nt-2294 nt). Poly(A)⁺ RNAs were separated on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane (Gen Screen Plus ; NEN). The RNA on the membrane was hybridized and washed under same conditions as for plaque hybridization. Panel (a) shows Northern blot analysis of the Poly(A)⁺ RNAs (2.5 μ g) from several tissues; lane 1, liver; lane 2, brain; lane 3, lung; lane 4, kidney. Panel (b) shows Northern blot hybridization of the Poly(A)⁺ RNAs (1 μ g) from peripheral blood monocytes. lane 1, with 50 μ g/ml LPS; lane 2, without LPS.

expression in bovine cells and tissues will facilitate the understanding of endotoxemia and other related diseases of cattle .

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