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Hepatocytes Contribute to Soluble CD14 Production, and CD14 Expression Is Differentially Regulated in Hepatocytes and Monocytes*

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CD14 presents as a glycosylphosphatidylinositol-linked membrane protein on the surface of monocytes/macrophages and as a soluble protein in the serum. Our previous studies have shown that an 80-kilobase pair (kb) genomic DNA fragment containing the human CD14 gene is sufficient to direct CD14 expression in a monocyte-specific manner in transgenic mice. In addition, we discovered that human CD14 is highly expressed in hepatocytes. Here, we report the generation of transgenic mice with either a 24- or 33-kb human CD14 genomic DNA fragment. Data from multiple transgenic lines show that neither the 24- nor the 33-kb transgenic mice express human CD14 in monocytes/macrophages. However, human CD14 is highly expressed in the liver of the 33-kb transgenic mice. These results demonstrate that human CD14 expression is regulated differently in monocytes and hepatocytes. Furthermore, we identified an upstream regulatory element beyond the 24-kb region, but within the 33-kb region of the human CD14 gene, which is critical for CD14 expression in hepatocytes, but not in monocytes/macrophages. Most importantly, the data demonstrate that the liver is one of the major organs for the production of soluble CD14. These transgenic mice provide an excellent system to further explore the functions of soluble CD14.

Every year septic shock promoted by Gram-negative bacteria causes over 100,000 deaths in the United States (1, 2). Lipopolysaccharide (LPS),[1] an endotoxin of Gram-negative bacteria, is known to be responsible for initiating host responses leading to septic shock (3). LPS stimulates its response by inducing the host cells to produce and release endogenous mediators including the proinflammatory cytokines interleukin-1, interleukin-6, and tumor necrosis factor-α (4). Lipopolysaccharide-binding protein (LBP), CD14, and Toll-like receptors (TLRs) are mediators of LPS stimulation (5–10). There are two forms of CD14. One is the glycosylphosphatidylinositol-anchored membrane CD14 (mCD14) found mainly on the surface of cells of myeloid lineage. The other is soluble CD14 (sCD14), found in the serum and urine (11, 12). LPS binds to CD14 in the presence of an acute phase response protein LBP. This complex mediates sepsis through TLRs. The role of CD14, LBP, and TLRs in LPS signaling has been well supported by studies in animal models (13–16). Besides its function in endotoxin signaling, it has been proposed that CD14 is involved in transportation of other lipids (17, 18), cell-cell interaction during different immune responses (19–21), and recognition of apoptotic cells (22, 23). Therefore, CD14 becomes an interesting molecule to investigate in various biological processes.

CD14 is highly expressed on the surface of monocytes/macrophages and strongly up-regulated during the differentiation of monocytic precursor cells into mature monocytes (24–26). Therefore, CD14 has been commonly used as a differentiation marker for monocytes/macrophages. CD14 serves as an excellent model for the study of monocytic gene regulation and lineage differentiation. We have previously reported that tissue-specific CD14 expression occurs at the level of transcription (27, 28). Furthermore, two transcription factors, Sp1 and C/EBP, play critical roles in the activity of the CD14 promoter (27, 29). Using an 80-kb human CD14 genomic DNA fragment in transgenic animal studies, we have demonstrated that this fragment contains critical regulatory elements which direct CD14 gene expression in the monocytic lineage (30). In addition, we discovered the clear expression of human CD14 in the hepatocytes of 80-kb human CD14 transgenic mice as well as in human liver tissue and hepatocytic cell lines (30). These finding have been further supported by a recent report by Su *et al.* (31) that hepatocytes prepared from normal human liver have high CD14 expression.

To further elucidate the molecular mechanism of CD14 gene regulation, we generated transgenic mice with two smaller genomic fragments, which contain the human CD14 gene. One is 24 kb, and the other is 33 kb in length. Both are within the above mentioned 80-kb CD14 fragment. Data collected from multiple transgenic founder lines indicated that the expression of human CD14 is differentially regulated in monocytic and hepatocytic lineages. The 24-kb fragment did not show any human CD14 expression in transgenic mice. The 33-kb fragment did not show human CD14 expression in monocytic cells, but showed a high level of CD14 expression in a copy number-dependent and position-independent manner in the livers of transgenic mice. Using DNase I-hypersensitive site analysis and transient transfection studies, we were able to localize a tissue-specific distal regulatory element for CD14 expression in hepatocytes. Moreover, we revealed that the liver is one of the major sources of soluble CD14 production.

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[1] The abbreviations used are: LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay; kb, kilobase pair(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LBP, lipopolysaccharide-binding protein; TLR, Toll-like receptor.

MATERIALS AND METHODS

Generation of Transgenic Mice—The P1 phagemid containing the human CD14 genomic sequence (P1-CD14) was described previously (30). A 24-kb *Bam*HI fragment and a 33-kb *Kpn*I fragment of P1-CD14 (Fig. 1) were prepared for microinjection by gel electrophoresis and subsequent extraction with a Geneclean kit (Bio 101, Vista, CA) following *Bam*HI or *Kpn*I digestion and prepared as described previously (30). Transgenic mice were produced in the transgenic facility of Beth Israel Deaconess Medical Center using zygotes from FVB/N mice.

Southern Blot Analysis—Murine genomic DNA was prepared and analyzed as described previously (30). The relative copy number of the transgene was estimated by comparing the transgenic murine tail DNA samples using [^{32}P]dNTP-labeled human or murine CD14 cDNA fragments using ImageQuant software from Molecular Dynamics.

Isolation of RNA and Northern Blot Hybridization—Total RNA was isolated as described previously (28). The purified RNA samples were denatured in a formamide/formaldehyde solution, followed by electrophoresis on a 1% agarose gel containing 0.22 M formaldehyde. The RNA was then transferred to positively charged Biotrans nylon membrane (ICN, Costa Mesa, CA) and hybridized with human CD14 cDNA or GAPDH cDNA, which was labeled with [^{32}P]dCTP by random priming. Autoradiography was performed at −80 °C with Kodak BioMax MR film. The level of human CD14 expression relative to GAPDH was calculated using ImageQuant from Molecular Dynamics.

DNase I-hypersensitive Site Analysis—The preparation of nuclei from mouse liver was described previously (32). The nuclei were suspended in buffer D (100 mM NaCl, 50 mM Tris-HCl, pH 8.0, 3 mM $MgCl_2$, 0.4 mM phenylmethylsulfonyl fluoride, and 1 μg/ml each of antipain, chymostatin, leupeptin, and pepstatin A), followed by DNase I treatment as described previously (33). Ten μg of liver DNA subjected to DNase I treatment for varying lengths of time were digested by *Sac*I, electrophoresed on a 0.8% agarose gel, transferred to positively charged Biotrans nylon membrane (ICN, Costa Mesa, CA), and then hybridized with a radiolabeled 1.2-kb *Sac*I-*Hinc*II CD14 genomic DNA fragment 9 kb to 7.8 kb upstream of transcription initiation site (Fig. 4*A*).

Plasmid Construction—pXP2 is a promoterless luciferase construct (34). The construction of p-227CD14-luc was described previously (27). A 4.2-kb *Bgl*II/*Bam*HI fragment, a 3-kb *Sac*I/*Bam*HI fragment, and a 0.7-kb *Sac*II/*Bam*HI fragment, which are 6 kb upstream from the human CD14 transcription initiation site, were inserted into the *Bam*HI site of p-227CD14-luc in sense (s) and antisense (a) orientations, and were named as p-4.2K(s)227CD14-luc and p-4.2K(a)227CD14-luc, p-3K(s)227CD14-luc and p-3K(a)227CD14-luc, p-0.7K(s)227CD14-luc and p-0.7K(a)227CD14-luc (Fig. 5).

Cell Culture—Human myeloblastic U937 cells and human cervical carcinoma HeLa were cultured as described previously (35). Human hepatoma HepG2 cells was cultured in Dulbecco's modified Eagle's medium (BioWhittaker, Walkersville, MD) supplemented with 10% calf serum (Sigma) and 2 mM L-glutamine Human Mono Mac 6 cells were propagated as described (36).

Transient Transfection—HepG2 or HeLa cells (4×10^4) were plated 24 h prior to transfection into each well of a 24-well plate. They were transiently transfected with 1 μg of the reporter constructs as indicated in the figures, and 1 ng of pRL-CMV as an internal control using LipofectAMINE Plus (Life Technologies, Inc.) following the manufacturer's protocol. The cells were harvested 16–20 h after transfection in 100 μl of lysis buffer, and luciferase assays were performed using the dual luciferase system (Promega, Madison, WI) following the manufacturer's instructions. Mono Mac 6 cells were transfected as described previously with 20 μg of the reporter constructs and 10 ng of pRL-CMV as an internal control (27). The cells were harvested 5 h after transfection in 100 μl of lysis buffer, and luciferase assays were performed as above.

Serum Collection and Human CD14 ELISA—Murine sera were collected via tail bleeding, followed by centrifugation at $325 \times g$ for 10 min at 4 °C. ELISA plates were coated with 1 μg/ml monoclonal anti-human CD14 antibody (28C5). The samples, as well as a recombinant human CD14 standard, were incubated, and bound human CD14 was detected using a biotinylated anti-human CD14 antibody (18E12). The antibodies and recombinant human CD14 were kindly provided by Dr. P. S. Tobias of the Scripps Research Institute.

RESULTS

Generation of Transgenic Mice—Previous studies have demonstrated that the Sp1 sites and C/EBP site in the proximal promoter region of the CD14 gene are critical for CD14 expression (27, 29). Furthermore, the C/EBP site mediates TGFβ signaling during monocyte differentiation. The data also show that an 80-kb genomic DNA fragment including the human CD14 gene provides tissue-specific CD14 expression in monocytes/macrophages and in the liver of transgenic mice (30). To further understand the molecular basis of CD14 expression, we isolated a 24-kb *Bam*HI CD14 genomic DNA fragment and a 33-kb *Kpn*I CD14 genomic DNA fragment and used them to generate transgenic mice (Fig. 1). Five founder lines of 24-kb transgenic mice and six founder lines of 33-kb transgenic mice were obtained. All five founder lines of 24-kb transgenic mice and four of six founder lines of 33-kb transgenic mice were germline transmitted. These founder lines, which exhibited germline transmission of the transgene, were used in further investigations.

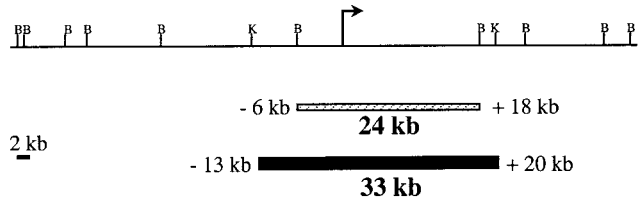

FIG. 1. **Physical map of the P1 phagemid clone, which contains the human CD14 gene.** The P1 clone contains ~40 kb of upstream sequence and ~40 kb downstream sequence of the CD14 gene. The *arrow* indicates CD14 gene transcription initiation site. The 24-kb *Bam*HI fragment and 33-kb *Kpn*I fragment that contain the human CD14 gene were used to generate transgenic mice. *B*, *Bam*HI digestion site; *K*, *Kpn*I digestion site.

Tissue-specific Expression of Human CD14 in Transgenic Mice—In the 80-kb transgenic mice, human CD14 was highly expressed in peritoneal macrophages and liver, which is consistent with its expression pattern in human macrophage and liver (30). To investigate the expression of human CD14 in the 24- and 33-kb transgenic mice, RNA was prepared from various tissues of these transgenic mice. Northern hybridization analyses with human CD14 as a probe showed that there was no highly detectable human CD14 expression in any of the tested tissues from the 24-kb transgenic mice (data not shown). However, human CD14 was highly expressed in the liver and mildly expressed in the heart, thymus, and lung of the 33-kb transgenic mice (Fig. 2*A*). There is no highly detectable human CD14 expression in the macrophages of the 33-kb transgenic mice. To further verify the relative level of CD14 expression in the liver, heart, thymus, and lung in the transgenic mice, RNA samples prepared from these tissues were analyzed again with a more evenly loaded gel (Fig. 2*B*). These results indicate that liver is the major organ for human CD14 expression in 33-kb transgenic mice; lung also expresses significant amounts of CD14. All four founder lines showed a similar pattern of human CD14 expression. Comparing this with the results from the 80-kb transgenic mice, the data indicate that regulatory elements beyond 33 kb, but within the 80-kb flanking regions of human CD14, are necessary for human CD14 expression in macrophages. The results also suggest that the regulatory elements involved in human CD14 expression in macrophages and liver are different, and that the region located outside of 24-kb but within 33-kb flanking regions of human CD14 gene contains important regulatory element(s) for human CD14 expression in the liver.

Expression of Human CD14 in Liver Is Copy Number-dependent and Position-independent—To study the regulation of CD14 expression in 33-kb human CD14 transgenic mice, we first analyzed the copy number of the human CD14 fragment in four germline transmitted founder lines using Southern blot hybridization (Fig. 3*A*). We then studied the level of human CD14 expression in four founder lines using Northern blot

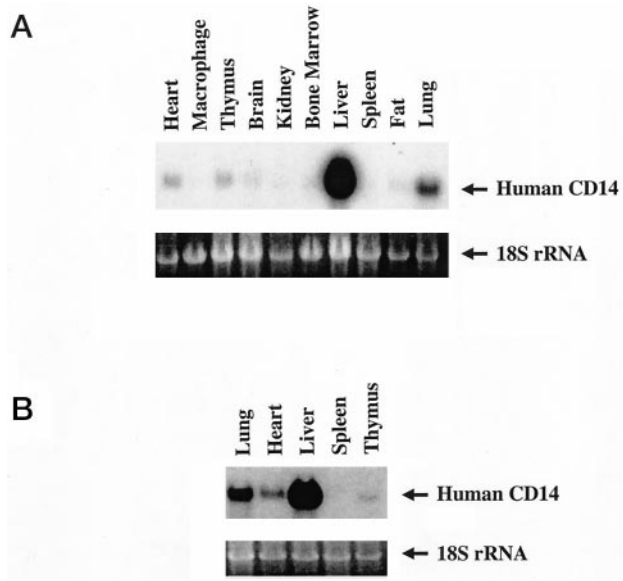

FIG. 2. **Northern blot analysis of human CD14 expression in various tissues from founder line 39.** Ten-μg RNA samples prepared from different tissues of the transgenic mice were electrophoresed on a 1% agarose gel containing 0.22 M formaldehyde. The gel was transferred to a positively charged nylon membrane and hybridized with a human CD14 cDNA probe. The ethidium bromide staining of the 18 S ribosomal RNA is presented to show the loading of the RNA samples.

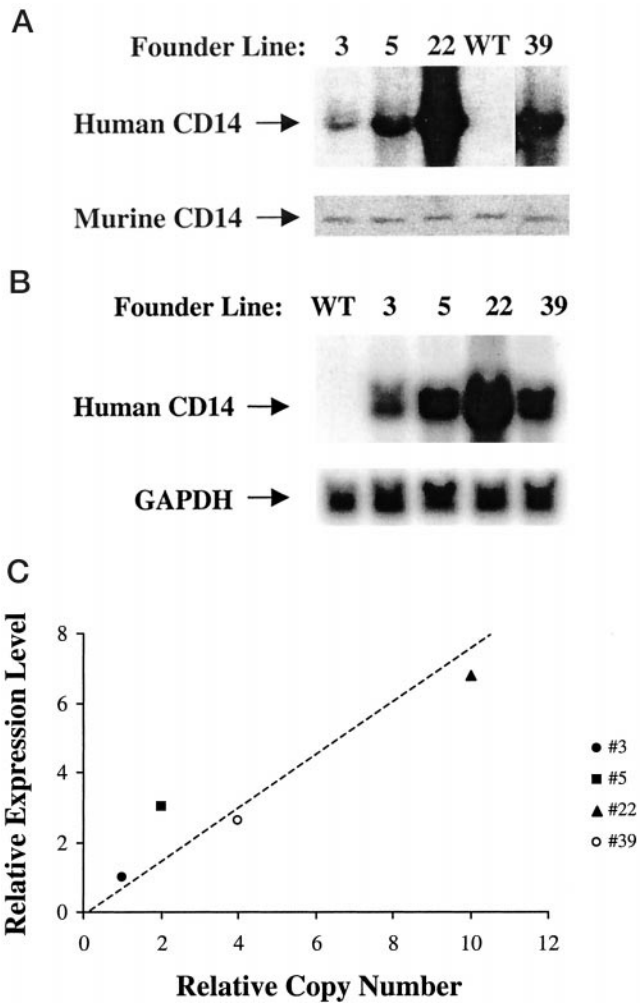

FIG. 3. *A*, Southern blot analysis of human CD14 transgene in different transgenic founder lines. Ten μg of DNA prepared from each founder line were electrophoresed on a 1% agarose gel, transferred to nylon membrane, and sequentially hybridized to human and murine CD14 cDNAs. The murine CD14 hybridization is to confirm the relative equal loading of DNA from various founder lines. *B*, Northern blot analysis of human CD14 expression in livers of transgenic mice from different founder lines. A wild type mouse and mice from different founder lines were euthanized, and total RNA was isolated from the livers. The RNA samples (10 μg each) were then electrophoresed on a 1% agarose gel containing 0.22 M formaldehyde. The gel was transferred to a positively charged nylon membrane that was subsequently hybridized with a human CD14 cDNA probe. The expression of GAPDH is presented to show the loading of the RNA samples. *C*, correlation of the expression level and the copy number of human CD14 in different transgenic founder lines. The relative amounts of human CD14 DNA and the relative expression levels of human CD14 shown in *panels A* and *B* were quantified using a phosphorimager and ImageQuant software from Molecular Dynamics. *#3*, *#5*, *#22*, and *#39* represent various founder lines. *WT* represents samples from wild type mice.

hybridization with RNA prepared from these transgenic mice (Fig. 3*B*). Densitometry analysis of the results revealed that human CD14 expression levels relative to GAPDH expression in different founder lines and their copy numbers have a linear relationship (Fig. 3*C*). This indicates that human CD14 expression in the 33-kb transgenic mice is copy number-dependent. Since the integration of the 33-kb fragment is random in the murine genome, the data also suggest that the expression of human CD14 in the liver of transgenic mice is position-independent.

A Distal Positive Regulatory Element Was Identified for Human CD14 Expression in Hepatocytes—To further identify the regulatory elements within the 33-kb region of human CD14, a DNase I-hypersensitive site study was performed. We developed a 1.2-kb probe located at the 5′ end of a *Sac*I-digested fragment (Fig. 4*A*) and applied this probe to *Sac*I-digested DNA from the livers of transgenic mice following DNase I treatment of the liver nuclei for varying lengths of time. The results revealed two DNase I-hypersensitive sites within the 33-kb region. One site is 6.3 kb, and another is 7 kb upstream of the transcription initiation site of the human CD14 gene (Fig. 4, *A* and *B*).

To investigate the function of the region containing DNase I-hypersensitive sites for CD14 expression, a 4.2-kb *Bgl*II -*Bam*HI fragment as shown in Fig. 4*A* was subcloned into a human CD14 proximal promoter-luciferase reporter construct p-227CD14-luc (27). We made luciferase constructs, which included the 4.2-kb *Bgl*II-*Bam*HI fragment in either sense orientation (p-4.2K(s)227CD14-luc) or antisense orientation (p-4.2K(a)227CD14-luc) (Fig. 5). With the CD14 proximal promoter-luciferase construct (p-227CD14-luc) as a control, these constructs were transiently transfected into a hepatocytic cell line, HepG2; a monocytic cell line, Mono Mac 6; and a cervical carcinoma cell line, HeLa. The results showed that the 4.2-kb fragment enhanced the activity of the CD14 proximal promoter in HepG2 cells by 25-fold when it is in the sense orientation, but only weakly enhanced the activity in the antisense orientation. Furthermore, it did not significantly enhance the promoter activity in either orientation in Mono Mac 6 cells or HeLa cells (Fig. 5). These results indicate that this 4.2-kb fragment contains distal regulatory element(s), whose activity is orientation-dependent and tissue-specific.

Deletion constructs from the 4.2-kb fragment were generated to further study the regulation of CD14 expression in hepatocyte. As shown in Fig. 6, the sense oriented 4.2-kb *Bgl*II-*Bam*HI fragment, 3-kb *Sac*I-*Bam*HI fragment, and the 0.7-kb *Sac*II-*Bam*HI fragment had similar positive effect on the CD14 proximal promoter in HepG2 cells. The data indicate that the positive distal regulatory element is located in the 0.7-kb fragment. Furthermore, the 4.2- and 3-kb fragments showed strong orientation dependence, and the 0.7-kb fragment only showed partial orientation dependence.

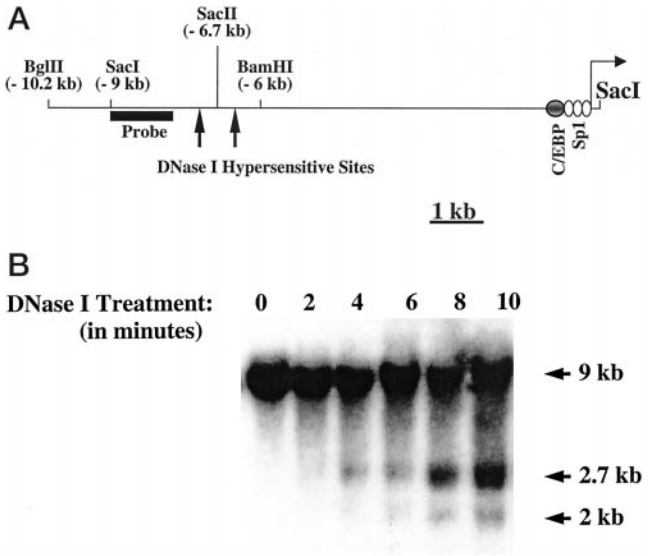

FIG. 4. *A*, schematic diagram of the upstream region of the human CD14 gene. The DNA probe is indicated by the *filled box*. The C/EBP and Sp1 binding sites in the proximal promoter region are shown, as well as the transcription initiation site of human CD14 (*horizontal arrow*). The DNase I-hypersensitive sites were located 6.3 and 7 kb upstream from the human CD14 transcription initiation site (*vertical arrows*). Restriction enzyme digestion sites used to produce regulatory fragment-luciferase constructs were indicated. *B*, DNase I-hypersensitive site analysis of the region upstream from the human CD14 gene in liver. Genomic DNA was isolated from the nuclei of liver cells from a transgenic mouse of founder line 3 following DNase I treatment for various times as indicated. After *Sac*I digestion, 10 μg of genomic DNA from each time point were electrophoresed in a 0.8% agarose gel, transferred to a positively charged nylon membrane, and hybridized with the probe indicated in *panel A*. Two DNase I-hypersensitive sites were detected. The sizes of the fragments are indicated on the *right side* of the panel.

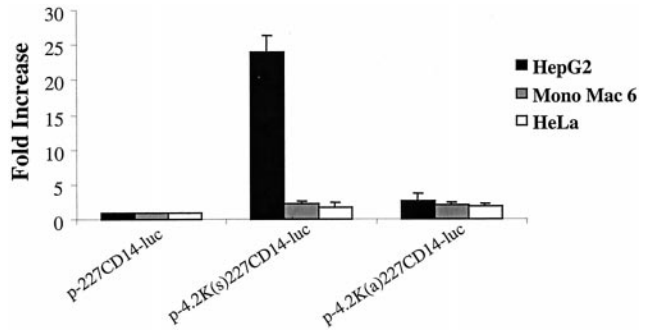

FIG. 5. **Identification of distal regulatory elements in the upstream region containing DNase I-hypersensitive sites.** The human CD14 promoter-luciferase construct (p-227CD14-luc) and the constructs containing a 4.2-kb *Bgl*II/*Bam*HI region with the DNase I-hypersensitive sites in sense orientation (p-4.2K(s)227CD14-luc) or antisense orientation (p-4.2K(a)227CD14-luc) were transiently transfected into HepG2, Mono Mac 6, and HeLa cells. Luciferase activities were normalized by cotransfecting with a *Renilla* luciferase construct (pRL-CMV) as an internal control. The relative luciferase activities of the constructs were averaged together from three separate sets of experiments. The *error bars* represent the standard deviations.

Liver Is One of the Major Sources of Soluble CD14—Soluble CD14 is able to mediate LPS signaling and initiate the cytokine cascade in normal human monocytes (37), as well as in cells that lack membrane-bound CD14, such as epithelial and endothelial cells (38, 39). Furthermore, soluble CD14 has a potential involvement in other lipid transfer processes (17). As shown in the above sections, the human CD14 gene is clearly expressed in the livers of 33-kb transgenic mice although it is not expressed in the monocytes/macrophages of these transgenic mice. To further study the biological significance of our transgenic mice, we investigated human CD14 expression at the protein level in the liver and serum of four transgenic founder lines. Due to the high background of liver proteins with human CD14 antibodies, we were unable to clearly detect the CD14 protein in liver protein extracts by Western blot analysis (data not shown). However, when an ELISA was used to analyze the level of soluble human CD14 in the serum of transgenic mice, we detected a clear expression of human CD14 (Fig. 7). Furthermore, the amount of soluble human CD14 expression had a direct correlation with the copy number of the human CD14 transgene in the transgenic mice. These data indicate that liver is one of the major tissues in which soluble human CD14 is produced.

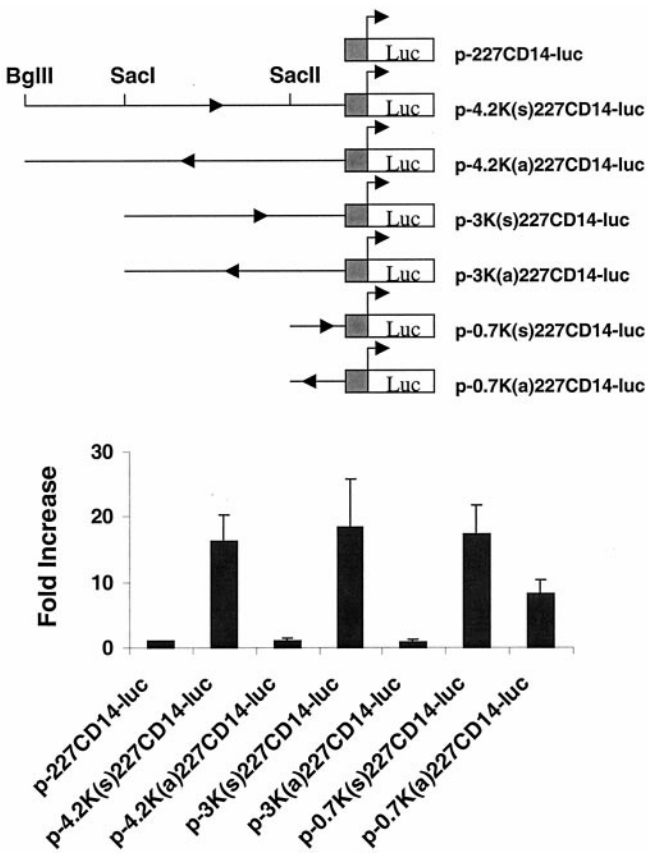

FIG. 6. **Deletion analysis of distal regulatory elements within 4.2 kb of *Bgl*II/*Bam*HI region.** The human CD14 promoter-luciferase construct (p-227CD14-luc) and the constructs containing 4.2-kb *Bgl*II/*Bam*HI region, 3-kb *Sac*I/*Bam*HI region, and 0.7-kb *Sac*II/*Bam*HI region in sense or antisense orientation were transiently transfected into HepG2 cells. Luciferase activities were normalized by cotransfecting with a *Renilla* luciferase construct (pRL-CMV) as an internal control. The relative luciferase activities of the constructs were averaged together from three separate sets of experiments. The *error bars* represent the standard deviations.

DISCUSSION

Since CD14 is specifically expressed in monocytic cells during hematopoiesis and has been used as a differentiation marker for monocytes/macrophages, our original goal was to study the expression of CD14 in the monocytic lineage in order to gain information about myeloid cell differentiation. However, in the analysis of our 80-kb transgenic mice and subsequent investigation with human tissues and cell lines, we have demonstrated a strong human CD14 expression in both monocytic cells and in hepatocytes (30). Using 24- and 33-kb CD14 genomic DNA fragments in current studies in transgenic mice, we have further revealed the differential regulation of CD14 expression in monocytic and hepatocytic cells. The data indi-

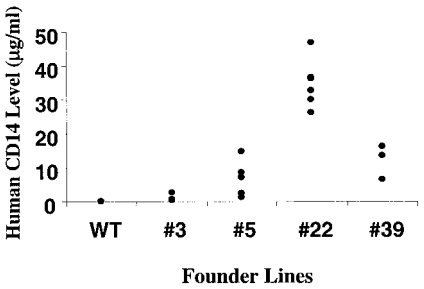

FIG. 7. **Human CD14 concentration in sera of wild type mice and different founder lines of transgenic mice by ELISA.** The sera from wild type mice ($n = 2$), as well as from transgenic mice from founder line 3 ($n = 3$), founder line 5 ($n = 5$), founder line 22 ($n = 6$), and founder line 39 ($n = 3$), were analyzed by ELISA with a monoclonal antibody against human CD14. The levels of human CD14 are shown in the *graph*.

cate that the 24-kb DNA fragment lacks important regulatory elements to support CD14 expression. The sequence within the 33-kb fragment, but beyond the 24-kb fragment, is required for directing CD14 expression in a copy number-dependent and position-independent manner in hepatocytes; and monocytic CD14 expression requires further genomic information beyond the 33-kb fragment. Using DNase I hypersensitivity and transfection studies, we have identified a 0.7-kb DNA fragment within the 33-kb genomic sequence, which functions as a hepatocyte-specific regulatory element. Therefore, we have generated an animal model, which exhibits human CD14 expression in the liver but not the monocytes.

It is interesting to note that the critical transcription factors regulating CD14 promoter activity are Sp1 and C/EBP (27, 29). Sp1 is a ubiquitously expressed transcription factor (40). It directly interacts with basal transcription machinery, such as binding to TAF110, and cooperates with tissue-specific factors to promoter gene expression (41). C/EBPs are a family of transcription factors (42). Some of the family members are expressed in a tissue-specific fashion, and others are expressed during cell stress and acute phase response. Among these members, C/EBPα and C/EBPβ are highly expressed in both hepatocytes and myeloid cells (43). Mice with C/EBPα or C/EBPβ deficiency have shown significant impairment in the differentiation or function of these cells (44–46). Therefore, hepatocytes and monocytes share the same set of transcription factors and use the same CD14 upstream region as their proximal promoter. The different regulation of their expression in two cell types depends on further upstream regulatory elements. Two DNase I-hypersensitive sites were identified by a DNase I hypersensitivity study at 6.3 and 7 kb upstream from the human CD14 gene. Transient transfection experiments in a hepatocytic cell line (HepG2), a cervical carcinoma cell line (HeLa), and a monocytic cell line (Mono Mac 6) show that a 4.2-kb (*Bgl*II/*Bam*HI) fragment, including these two hypersensitive sites, enhances the proximal CD14 promoter activity in hepatocytes, but not in endothelial cells and macrophages. This indicates the tissue specificity of this regulatory element, which is consistent with the results from transgenic mice. This also demonstrates that human CD14 expression in the liver is due to its expression in hepatocytes instead of cells from the liver macrophage lineage, Kupffer cells. As shown in Fig. 5, the positive effect of 4.2-kb upstream fragment has an orientation preference. To further understand the mechanism of such orientation dependent regulation, we used four additional constructs containing two smaller fragments in both orientations and used in the transient transfection assay. The 3-kb (*Sac*I/*Bam*HI) fragment showed the same effect as 4.2-kb fragment. The 0.7-kb (*Sac*II/*Bam*HI) fragment has the same positive effect in the sense orientation and a lower, but substantial positive effect in the antisense orientation. These results indicate that a hepatocyte-specific enhancer sequence is located within the 0.7-kb DNA fragment. Furthermore, a regulatory element surrounding *Sac*II site may function as an insulator for establishing an independent CD14 regulatory domain in hepatocytes.

Prompted by the analysis of results seen in transgenic mice, we found that human CD14 is highly expressed in the liver (30). These results showing differentially regulated CD14 expression in the two cell lineages have led us to search the literature for studies concerning regulated CD14 expression in the liver. Volpes *et al.* (47) briefly reported their observation in 1991 that CD14 expression can be detected on the surface of hepatocytes in liver allograft rejection patient samples, but not on hepatitis specimens using the anti-CD14 monoclonal antibodies LeuM3 and WT14. Fearns *et al.* (48) reported detectable CD14 expression in mouse liver after LPS stimulation. Recently, studies with rat and human primary hepatocytes and hepatocytic cell lines revealed additional information about CD14 basal expression and up-regulation by LPS (49, 50). Since the liver is the major organ involved in acute phase response and CD14 is directly responsible for the sensitive signal transduction of the endotoxin LPS, it is important to study both the regulation of liver CD14 expression in LPS signaling and CD14 function during LPS signaling. The generation of these transgenic mice provides a good animal model system for these analyses.

In the 33-kb transgenic mice, human CD14 is highly expressed in the liver, but not in macrophages. Human soluble CD14 are detected in these transgenic mice. Although human CD14 expression is also detected in the lung, heart, and thymus, its expression in the liver is much higher and liver mass is much bigger than the other tissues. This indicates that the expression of human CD14 in the liver generates soluble CD14. More importantly, this shows for the first time *in vivo* that the liver is one of the major sources of soluble CD14 in circulation. The soluble CD14 level in the serum of normal adult human is about 5 μg/ml, which represents approximately a 1000-fold molar excess of the LPS level seen in fatal septic shock patients (17). This indicates that soluble CD14 may have other biological functions besides its function in LPS signaling. Both membrane-bound and soluble forms of CD14 have been recently reported as potential lipid transport proteins (17, 18). Level of soluble CD14 in normal mouse serum is much lower than in human serum (51). We have generated transgenic mice, which express different levels of soluble CD14. Some of these transgenic lines have soluble human CD14 expression at the similar level to CD14 in human sera. They should be good models for analyzing CD14 biological functions.

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