Identification of a Tissue-specific Regulatory Element within the Murine CD14 Gene*

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We previously isolated and sequenced the 5'-flanking region of the mouse CD14 (mCD14) gene (Matsuura, K., Setoguchi, M., Nasu, N., Higuchi, Y., Yoshida, S., Akizuki, S., and Yamamoto, S. (1989) Nucleic Acids Res. 17, 2132). To define the regulatory elements that control expression of the mCD14 gene, we analyzed the structure of the 5' end of the gene, including a region further upstream of that determined previously. Sequentially 5'-deleted, chimeric, and point mutated clones were tested for the ability to stimulate chloramphenicol acetyltransferase. An 8-base pair sequence, TGATTCCAC, at position -255, which resembled the consensus sequence of the 12-O-tetradecanoylphorbol-13-acetate-responsive element (TRE), enhanced the expression of the chloramphenical acetyltransferase gene in macrophage (aHINS-B3) and non-macrophage (glioblastoma G203 and myeloma NS1) cells. The enhancing ability of the TRE-like sequence (TLS), however, was markedly reduced in G203 cells but not in aHINS-B3 cells when the TLS was followed by the sequence immediately downstream. The TLS and sequence immediately downstream were capable of binding nuclear proteins which were unique to aHINS-B3 cells and macrophages, suggesting that these unique protein regulate the specific expression of the mCD14 gene. Binding of AP-1 to the TLS was also found in aHINS-B3 and G203 cells. Although it is uncertain whether AP-1 is involved in expression of the mCD14 gene, the effect of AP-1 in non-macrophage cells was inhibited by a nuclear protein which binds to the sequence immediately downstream of the TLS.

CD14 is a myelomonocytic differentiation antigen (1-7) and is the cell surface receptor for the lipopolysaccharide-lipopolysaccharide-binding protein complex (8). Interaction of CD14 with the complex activates myelomonocytic cells, leading to tumor necrosis factor production (9).

Cell type-specific regulation of gene transcription appears to be mediated by the binding of regulatory proteins (trans-acting factors) to specific cis-regulatory regions of genes that are usually, but not always, located in the 5'-flanking region of the gene. Cis-regulatory regions usually consist of short DNA binding sites and a combination of binding sites for ubiquitous and cell-specific factors. Expression of the CD14 gene is highly tissue-specific. It is, therefore, of interest to identify the specific cis-regulatory regions and trans-acting factors that are responsible for the expression of this gene and to assess of their degree of tissue specificity.

We already cloned the mCD14 gene and 5'-flanking region (10, 11). To begin to characterize the cis- and trans-acting elements involved in the transcriptional control of the mCD14 gene, we analyzed the upstream region of the mCD14 gene. We here describe potential cis-acting regulatory elements in the 5'-flanking region, show that an 8-base pair upstream sequence confers cell type-specific expression onto a reporter gene in an mCD14-positive macrophage cell line, and suggest that unique positive regulatory elements are required to achieve cell-specific expression of the mCD14 gene.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes were purchased from Takara, Toyobo, Resheeda Research Laboratories, and Wako Pure Chemicals. Agarose, ultrapure DNA grade, and DNA ligation kit were from Takara. A 5’ deletion kit was obtained from Nippongene. Radioactive nucleotides [α-32P]dCTP (3000 Ci/mmol) and [γ-32P]ATP (6000 Ci/ mmol) were obtained from Du Pont-New England Nuclear. Chemicals used for DNA sequencing were obtained from Toyobo; X-ray film (XAR-351) was obtained from Kodak.

Cell Lines, Cells, and Cell Cultures—Murine macrophage cell lines aHINS-B3 (12-14) and J774, murine glioblastoma line G203, kindly supplied by Dr. J. Kuratsu (Department of Neurosurgery, Kumamoto University), and the murine myeloma cell line P3/NS1/1-Ag-1 (NS1) were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Murine peritoneal macrophages were obtained from the peritoneal cavity of ICR mice after injection with peptone (10%), 1 ml 4 days previously. Mouse liver, kidney, spleen, heart, and skeletal muscle were obtained from ICR mice.

Molecular Cloning and Sequencing—The 5'-upstream sequence up to -4 kilobases was cloned from a bacteriophage EMBL3 murine liver library (15) using [32P]-labeled cDNA containing the mCD14 insert (16, 17) and partially sequenced as described previously (12, 18).

S1 Nuclease Mapping—S1 nuclease mapping was carried out according to the method of Berk and Sharp (19). First, total RNA from aHINS-B3 cells was hybridized with an end-labeled Nhel-NcoI fragment (see Fig. 1) in 40 mM Pipes (pH 6.4), 0.4 mM NaCl, 1 mM EDTA, and 80% formamide for 16 h at 50°C. Following hybridization, the reaction was diluted 10-fold with S1 nuclease (60 units), and the reaction mixture was incubated for 35 min at 37°C. The reaction mixture was terminated by the addition of termination buffer (2.5 mM ammonium acetate and 50 mM EDTA), and the DNA-RNA hybrids were precipitated with ethanol, dissolved in the loading buffer, heated to 90°C, and resolved on a 6% acrylamide, 7 M urea sequencing gel.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) D19872.

1The abbreviations used are: mCD14, mouse CD14; Pipes, 1,4-piperazinediethanesulfonic acid; DTT, dithiothreitol; CAT, chloramphenicol acetyltransferase; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRE, TPA-responsive element; TLS, TRE-like sequence; IRE, interferon regulatory element; VDRE, vitamin D-responsive element-like sequence; ULR, upstream long repetitive.

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**Primer Extension Analysis**—The probe for primer extension analysis was a synthetic 20-base single-stranded end-labeled primer, 3′-AACAGGCCTCGGATGACCCG-5′ corresponding to base pairs 66-85 of the first exon of the mCD14 gene. Total cellular RNA was isolated from aHINS-B5 cells. The primer was annealed to 70 μg of total cellular RNA in the reaction mixture at 1 h at 37° C in 1× of annealing buffer (10 mM Tris-HCl (pH 8.3), 250 mM KCl, 1 mM EDTA), followed by incubation for 90 min at room temperature. The resulting DNA-RNA hybrid was precipitated in ethanol and dissolved in reverse transcriptase buffer (23.3 mM Tris-HCl (pH 8.3), 16.7 mM KCl, 13.5 mM MgCl2, 13.3 mM DTT), 1 mM dNTP, [α-³²P]ATP (5000 Ci/mmol), 200 units of reverse transcriptase, and 0.133 mM actinomycin D (Sigma). After 1 h at 37° C, the DNA-RNA hybrids were precipitated with ethanol, dissolved in loading buffer (95% formamide, 20 mM EDTA, 0.65% bromphenol blue, 0.05% xylene cyanol FF), heated to 90° C, and resolved on a 6% acrylamide, 8 M urea sequencing gel.

**Constructions of mCD14-Plasmids**—118pH10 clone containing the 739-base pair nucleotides corresponding to bases 678-866 of the 5′-upstream and exon 1 sequence of the mCD14 gene was digested with KpnI-SalI. The resulting insert was 5′-depleted using a kit (Takara) according to the manufacturer’s instructions. Briefly, the insert DNA was digested with exonuclease III. Aliquots (2.5 μl) were removed at 1-min intervals and mixed with 50 μl of 2× mung bean nuclease buffer. After heating for 5 min at 65° C, the samples were incubated with mung bean nuclease for 60 min at 37° C. The 5′-depleted samples were then digested with the Klenow fragment followed by treatment with T4 DNA polymerase to determine the extent of deletion into the 5′ end of the promoter, each mutant was sequenced as previously described (18). Selected mutants were digested with SacI and HindIII and inserted into the SacI-HindIII sites of the multicloning site containing pSVmCAT vector which was produced from the pSVOCAT vector. Plasmid DNA was prepared by alkaline lysis followed by two centrifugation steps through CsCl to isolate supercoiled plasmid DNA.

**DNA Transfections and CAT Assay**—Plasmid DNA was transfected into the mouse macrophage line aHINS-B5 and the mouse glioblastoma line G203 by calcium phosphate coprecipitation as described previously (20). Cells were transfected by the DEAE-dextran procedure essentially as described (20). After 4 h, the cells were given a 2-min glycerol shock followed by a wash with ice-cold phosphate-buffered saline. Fresh medium was then added and the incubation continued for 40-48 h. Cells were collected by centrifugation and suspended in 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA for 5 min on ice, harvested by scraping and centrifugation, and resuspended in 180 μl of 0.25 M Tris-HCl (pH 8.0). Extracts were prepared by freezing and thawing twice. The 10% cell lysate line NS1 was transfected by the DEAE-dextran procedure essentially as described by Grosschedl and Baltimore (20). Briefly, a total of 4×10⁶ cells were washed, resuspended, and incubated in 2 ml of 25 mM Tris, 137 mM NaCl, 5 mM KCl, 0.3 mM Na₂HPO₄, 2.7 mM KH₂PO₄, 1 mM MgCl₂, 1 mM CaCl₂, (pH 7.4) containing 0.5 mg/ml DEAE-dextran (Pharmacia LKB Biotechnology Inc.) and 10 μg of plasmid for 30 min at 37° C. After incubation, the cells were added with 5 ml of culture medium and incubated for 10 min at 37° C, then collected by centrifugation and suspended in 10 ml of medium. After 48 h in culture, cells were harvested with phosphate-buffered saline, freeze-thawed five times in 0.25 M Tris-HCl (pH 7.5), and heated to 65° C for 5 min to inactivate deacetylas. Supernatants were collected and assayed for protein according to the method of Bradford (21). CAT was assayed as described previously (22). The extract was incubated with [¹⁴C]chloramphenicol and 0.2 μg of actin-α, for 1 h at 37° C, and the products were separated by thin-layer chromatography.

**Oligonucleotides**—Complementary oligonucleotides CATCGGATCATCAATCAATCATCTAT (TLS-3), G(A/G)CCGATCATCAATCAATCATCTAT (TLS-4), GATACGCAATCATCAATCAATCATCTAT (TLS-5), GATACGCGATCATCAATCATCTAT (TLS-6), GATACGCGATCATCAATCATCTAT (TLS-7), GATACGCGATCATCAATCATCTAT (TLS-8), and AGTCGCGATCATCAATCATCTAT (TLS-9). PCR products were isolated from a 1% agarose gel. The oligonucleotides (10 μg of each strand) were annealed in a solution containing 50 mM Tris (pH 7.5), 10 mM MgCl₂, 50 μg/μl salmon sperm DNA, 10 mM EDTA, and 90° C for 2 min, and cooled at 65° C for 10 min, 37° C for 10 min, and room temperature for 5 min.

**Extract Preparation**—Nuclear extracts from a variety of cells were prepared as described by Schreiber et al. (23) with slight modification. Briefly, cells (approximately 2×10⁶) were harvested, pelleted, and suspended in ice-cold buffer (2 packed cell pellet volumes) containing 10 mM Hepes (pH 7.9 at 4° C), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM MgCl₂, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.3 μg/ml leupeptin, 0.3 μg/ml antipain, 0.3 μg/ml E64, 0.3 μg/ml pepstatin). After 15 min on ice, the cells were lysed with 25 μl of 10% Nonidet P-40 and centrifuged for 30 min in a microcentrifuge to pellet nuclei, the nuclear pellets were resuspended in ice-cold buffer containing 20 mM Hepes (pH 7.9), 0.5 mM NaCl, 1 mM EGTA, 1 mM EDTA, and antibiotics as above. After shaking for 15 min at 4° C, the nuclear extract was centrifuged for 5 min in a microcentrifuge and the supernatant was stored at -80° C.

**Gel Shift Assay**—Gel shift assays were performed as described with minor modifications (24). DNA probes consisted of synthetic oligonucleotides labeled with polynucleotide kinase and [³²P]ATP. DNA probes were incubated with protein fractions at room temperature for 30 min in 50 μl of binding reaction containing 10 mM Tris (pH 7.5), 50 mM NaCl, 0.62 mM ZnSO₄, 0.5 mM EDTA, 1 mM DTT, 5% glycerol, and -3 μg of poly(dl-dC) (Pharmacia). Binding reactions contained approximately 0.7 ng (1×10⁶ cpm) of probe and 40 μg of protein. Binding reactions were terminated with 5 μl of 50 mM EDTA containing 0.05% bromphenol blue, 0.05% xylene cyanol, and 5% glycerol. Mixtures were then electrophoresed on 5% acrylamide gels in 6.7 mM Tris (pH 7.9), 3.3 mM sodium acetate, and 1 mM EDTA at 4° C for 1-2 h at 170 V with buffer recirculation. Gels were transferred to filter paper, dried, and autoradiographed.

**RESULTS**

**DNA Sequence Analysis of the 5′ End of the mCD14 Gene**—We reported previously that the mCD14 gene (10) and the 5′-upstream sequence extended 623 nucleotides upstream of the initiation codon (position 1), which was defined by S1 nuclease mapping and primer extension analysis (Fig. 1, A and B). To analyze the cis-regulatory structure, a further 723 nucleotides upstream of the 5′ end sequence were sequenced (Fig. 24).

The 5′-upstream sequence, exon 1 and intron sequences (Fig. 2, A and B) contained a number of potential regulatory sequences. TATA box-like sequences (25) were found at positions -122 to -118 and -27 to -20. CAAT-like sequences (26, 27) were situated at positions -382 to -379, -127 to -124, and -75 to -72. The motif GGAAA and its invert TTTCC, was found at positions -696 to -692, -596 to -592, -396 to -392, -454 to -450, and -340 to -336, -314 to -310, and -245 to -240. This motif also resided in exon 1 at position 49-53. Interferon regulatory factor-1 (IRF-1) binding sequences AACTGA, AAGGGA, AAATGG, AAGTGA, AAGTGG, and AAGGGA and their inverted sequences TTACTA, were found at positions -493 to -488, -474 to -469, -411 to -396, -113 to -108, -85 to -80, -55 to -50, and -45 to -449, respectively. The TRE-like and TRE sequences, TGGTCA and TGATCA, respectively (31), were found at positions -255 to -249 and at the 5′ end of the intron respectively. The vitamin D-responsive element (VDRE)-like sequence (32) GGTTGAATCA was located at position -305 to -297.

A long repeat that had 90% homology with the nucleotide sequence at position -152 to 1 containing several TATA- and CAAT-like and IRF-1 binding sequences, was found at position -1285 to -1134. Alu-like B1-D2 and B1-C sequences were found at positions -1541 to -1005 and -799 to -704, respectively (33, 34).

**CAT Assay**—The Transcriptional Regulatory Regions Upstream of the mCD14 Gene—To delineate the regions essential for transcription of the mCD14 gene, we fused various portions of the 5′-flanking region to the bacterial CAT gene as a heterologous reporter gene. The deletions were generated by

**CD14 Promoter**
digestion of the HindIII-HindIII fragment of the mCD14 gene (118 pH10, 739 bases containing a promoter and an exon 1 region) with exonuclease III followed by mung bean nuclease digestion. This resulted in promoter fragments of 681, 541, 373, 364, 331, 318, 306, 260, and 109 base pairs. The HindIII-HindIII and deletion fragments were introduced into a CAT vector (Fig. 3; see Fig. 5). The constructs were transfected into G203 cells. The relative amount of reporter gene expression changed appreciably by progressively including additional 5' sequences up to -257 in aHINS-B3 cells but not in G203 cells. Further elongation of the 5'-upstream sequence diminished the reporter gene expression. These results indicate that the 5'-flanking region from positions -257 to -48, particularly -257 to -198, contains a cis-regulatory sequence specific for aHINS-B3 cells. To examine whether the upstream long repetitive sequence (ULR) had promoter activity similar to that of the downstream repeat, a CAT clone containing the XhoI-EcoRI fragment corresponding to positions -1308 to -1045 was constructed. The ULR, however, showed no CAT activity.

As stated above, the TLS was found at nucleotide position -256 to -249, which in the 5' region of 257CAT, demonstrated the highest CAT efficiency. We therefore predicted that TLS would play a role on the expression of the mCD14 gene. An oligonucleotide trimer with the sequence GATCCGTGATTCAGTGATTCAGATTCACTdesignated TLS-3 was synthesized and tested for CAT activity. TLS, when introduced upstream of the CAT gene in 48CAT, showed highly efficient CAT activity in aHINS-B3 cells (Fig. 4). The efficiency was also quite high in G203 (Fig. 4) and NS1 cells (data not shown). Similar results were obtained with the TLS-3R-CAT clone containing TLS-3 upstream of the CAT gene in 48CAT, in an inverted orientation. Specifically enhanced effect in aHINS-B3 cells with 257CAT suggested that a negative cis-regulatory element existed downstream of the TLS sequence. An oligonucleotide trimer with a sequence containing that immediately downstream of the TLS, designated TLSCT-3, was synthesized and examined for enhancing activity. CAT efficiency of TLSCT-3, when introduced upstream and downstream of the CAT gene in 48CAT, was high in aHINS-B3 cells (Figs. 4 and 5) but not in G203 (Fig. 4) and NS1 cells (data not shown). TLSCT-3 also had significant CAT activity in aHINS-B3 cells when inserted downstream of the CAT gene in 48CAT in an inverted orientation.

Point mutation of the first nucleotide sequence T to G of the TLS in 257CAT abolished enhancing activity. A similar finding has been reported for TSL (35). TLS is similar to the enhancer core sequence TGATTCAG in the glutathione transferase P gene, in which the core palindromic sequence is presented three nucleotides apart upstream (35, 36). Mutation of the eighth nucleotide, C to G, also greatly eliminated the activity in aHINS-B3 cells, suggesting that TLS is different from the enhancer sequence of the glutathione transferase P gene (Fig. 4). The mutation gave no effect on the activity in G203 cells although the level was low.

Gel Shift Analysis of Protein(s) That Specifically Bind to TLS and Its Downstream Sequence—To examine the specific binding ability of nuclear protein to TLS and its downstream sequence, band shift assay was performed. Three oligonucleotides GATCCCTGATTCACCTCCTAGTCCCTTTCCGGTTACT corresponding to positions -257 to -245, and -246 to -234, designated A and B, respectively, and TRE were used in competition experiments. Three distinct bands I, II, and III were identified with probe A when incubated with nuclear extract from aHINS-B3 cells (Fig. 6, A and B). Poly(dI-dC) was unable to inhibit the complex formation even at high concentrations (Fig. 6A). G203 nuclear extract formed a single band similar to band I, both of which were competed with TRE nucleotides (Fig. 6, B and D). Incubation of probe A with nuclear extracts from peritoneal macrophages,
**Fig. 2.** Structural organization of the 5'-flanking region of the CD14 gene. Panel A, nucleotide sequence of the 5' flanking region, exon 1 and intron of the mCD14 gene. Nucleotide positions are indicated at the left margin. The 5' end of the cDNA is designated as nucleotide 1 of the gene. Lower case indicates the intron. Regulatory elements, restriction sites, a few nucleotide positions, and the initiation methionine are indicated above the sequence. A triangle between CAAT and TATA indicates an SspI restriction site. The long underlines indicate long repetitive sequences. B1-D2 and B1-C are underlined with double lines and dashed lines, respectively. Panel B, schematic diagram of the 5'-flanking region and the mCD14 gene.

**Fig. 3.** Expression of 5'-deletion mutants and the mutant with the ULR sequence of the mCD14/CAT fusion gene in aHINS-B3 and G203 cells. A series of chimeric plasmids were constructed and transfected into aHINS and G203 cells. Filled columns are CAT activities in aHINS-B3 cells. Open columns are CAT activities in G203 cells. Nucleotide positions are indicated below the underline.
expression of chimeric plasmids was constructed and transfected into aHINS-B3 and G203 cells. CAT activity expressed in a transient assay was measured and is indicated as promoter activity relative to pSV2CAT. Filled columns are CAT activities in aHINS-B3 cells. Open columns are CAT activities in G203 cells. Nucleotide positions are indicated below the underline. > indicates a TLS. < indicates an inverted TLS. *< indicates an inverted TLSCAT. T → G indicates the 257CAT mutated at the first nucleotide of TLS, T for G. C → G indicates the 257CAT mutated at the eighth nucleotide of TLS, C for G.

FIG. 4. Effect of TLS-3, TLSCAT-3, and point mutation in TLS on expression of the CAT gene. A series of chimeric plasmids was constructed and transfected into aHINS-B3 and G203 cells. CAT activity expressed in a transient assay was measured and is indicated as promoter activity relative to pSV2CAT. Filled columns are CAT activities in aHINS-B3 cells. Open columns are CAT activities in G203 cells. Nucleotide positions are indicated below the underline. > indicates a TLS. < indicates an inverted TLS. *< indicates an inverted TLSCAT. T → G indicates the 257CAT mutated at the first nucleotide of TLS, T for G. C → G indicates the 257CAT mutated at the eighth nucleotide of TLS, C for G.

FIG. 5. Autoradiogram of CAT activity in extracts from aHINS-B3 cells transfected with various mCD14-CAT fusion genes. A series of chimeric plasmids was constructed and transfected into aHINS-B3 and G203 cells. CAT activity expressed in a transient assay was measured. pSV2CAT contains SV40 promoter only; 678CAT contains residues -678 to 61; 480CAT contains residues -48 to 61; 257CAT contains residues -257 to 61; 199CAT contains residues -199 to 61; 48CAT contains residues -48 to 61; TLSCAT-3-48CAT contains TLSCAT trimer sequence immediately upstream of the CAT gene in 48CAT; 48CAT-TLSCAT-3 contains TLSCAT trimer sequence immediately downstream of the CAT gene in 48CAT.

gonucleotide B appeared to be slightly higher than that of A. In addition, the formation of band II was significantly competed with TRE when a high concentration of TRE was included in the binding reactions. This may simply be attributable to that band II protein has affinity with TRE. Alternatively, it is possible to assume that band II protein has affinity with AP-1 since band I was effectively competed with B as well as TRE (Fig. 6B). Band III-forming protein, although the amount seemed less abundant, was also found in nuclear extracts from aHINS-B3 and macrophages but not in those from non-macrophage cells, suggesting that the protein also plays a role in CD14 expression.

A band corresponding to band II was formed with probe B when aHINS-B3, macrophage, and spleen nuclear extracts were used (Fig. 6C and Fig. 7, B, C, and E), whereas probe B formed another band (band IV) when G203, NS1, liver, and kidney cell nuclear extracts were used (Fig. 6D and Fig. 7, C and E). The band was discriminated from band II since it was exclusively formed with probe B. Gel shift assays with probe B using aHINS-B3 and macrophage nuclear extracts also showed that band I was not formed, suggesting that AP-1 released from band II-forming protein could not bind to probe B. These results are summarized in Table I.

DISCUSSION

Upstream of the mCD14 gene contains a variety of potential cis-regulatory elements including TATA- and CAAT-like sequences, IRF-1 binding sequences, a motif found in upstream sequences of interferon-inducible genes, VDRE-like sequence, as well as the TLS and TRE sequences. In addition, the repeated nucleotide sequence with the sequence at position -1 to -153 containing several TATA-, CAAT-like, and IRF-1 binding sequences was found at position -1285 to -1134. CAT analysis suggests that expression of the mCD14 gene is regulated by at least two 5'-upstream sequences; one is the proximal promoter element at position -48 to -1 containing a TATA-like sequence, and the other is the enhancer TLS at position -255 to -249. Since levels of enhancement by the proximal promoter element are significantly high in aHINS-B3 cells, the element would be necessary for expression of the mCD14 gene. TLS plays an important role in cell type-specific expression in aHINS-B3 cells. TLS-3 markedly enhances the level of expression of the CAT gene when inserted at the upstream of 48CAT in both orientations. A significant enhancement, however, was observed in G203. In contrast, TLSCAT-3 increased the level of CAT gene transcription when inserted at a different position or in inverse orientation in aHINS-B3 but not in G203 cells. Gel shift analysis showed that unique nuclear proteins in addition to AP-1 bound to TLS in aHINS-B3 cells. The major protein, which bound to TLS and the sequence immediately downstream, formed band II that was also found in macrophages, macrophage cell lines, and spleen but not in non-macrophage cells. In G203 cells, TLS was exclusively bound with AP-1. In addition, binding of another nuclear protein responsible for band IV to the sequence immediately downstream of TLS was observed. Nuclear proteins in other non-macrophage cells also contained
similar probe B-binding proteins. Band III-forming protein, which appeared to be less abundant in macrophages, may play a role in mCD14 expression in aHINS-B3 cells. Taken collectively, these results suggest that binding of unique nuclear proteins, especially band II-forming protein to TLS and the sequence immediately downstream, contributes to the specific expression of the mCD14 gene in monocytic lineages. TLS is capable of binding AP-1 in aHINS-B3 and G203 cells. Although it is uncertain whether AP-1 is involved in mCD14 gene expression, band IV-forming protein in G203 cells binds
Fig. 7. Gel shift and competition assays using A and B fragments and TRE with nuclear extracts from macrophages, macrophage and non-macrophage cell lines, and various organs. Panel A, gel shift and competition assays using A and B fragments and TRE with macrophage nuclear extracts. Panel B, gel shift and competition assays using A and B fragments with macrophage nuclear extracts. Panel C, gel shift assays using A and B fragments with aHINS-B3, macrophage, J774, and NS1 nuclear extracts. Panel D, gel shift assays using A fragment with nuclear extracts from macrophage, heart, liver, kidney, skeletal muscle, and spleen. Panel E, gel shift assays using B fragment with nuclear extracts from macrophage, heart, liver, kidney, skeletal muscle, and spleen. I, II, III, and IV denote the oligonucleotide-protein complexes by gel shift assays.
The 3' end of the sequence of TLS, C, is different from that the mCD14 gene caused the reduction of CAT expression. A palindromic sequence was found in the adjacent region upstream of the TLS in the mCD14 gene. It is, however, unlikely to G markedly reduces the expression, indicating that TLS is essential in the CD14 gene expression because mutation of C probably slight, if any. Importance of band 11- and IV-forming contribution of AP-1 on expression of the mCD14 gene is also suggested in other cells (Table I).

TTL, TGATTCCAC is similar to the enhancer core sequence, TGATTCCAG of the glutathione transferase P gene, (34) which is highly expressed in chemically induced rat hepatoma cells, precancerous hyperplastic nodules, and to a much lesser extent in other tissues including the lung, kidney, and testis. A palindromic sequence located 5'-upstream and situated 3' to immediately downstream of the TLS could inhibit the effect of AP-1. TPA, however, only had a marginal enhancing effect up to 60 min on expression of the mCD14 gene. The contribution of AP-1 on expression of the mCD14 gene is probably slight, if any. Importance of band 11- and IV-forming contribution of AP-1 on expression of the mCD14 gene is also suggested in other cells (Table I).

The enhancer effect of the GTP enhancer core and TRE sequence is reportedly abolished when the 5' end nucleus T to G. Similarly, a change in the 5' end of TLS in the mCD14 gene caused the reduction of CAT expression. The 3' end of the sequence of TLS, C is different from that of GTP enhancer core, which is G. It appears that C is essential in the CD14 gene expression because mutation of C to G markedly reduces the expression, indicating that TLS is a unique enhancer sequence although it is cross-reactive with AP-1 in addition to CD14-specific transcription factor(s).

Homology of the nucleotide sequence of the 5'-upstream repeat with the proximal promoter sequence reached 90%.

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TABLE I

Profiles of the formation of oligonucleotide-nuclear protein band complexes

| Types | Oligonucleotide probes | A     | B     |
|-------|------------------------|-------|-------|
| 1     | I, II, III             | II    | aHINS-B3 |
| 2     | II, III*               | II    | Macrophages, J774 |
| 3     | I                     | IV    | G203   |
| 4     | I*, II*               | IV    | N51    |
| 5     |                      | IV    | Kidney, liver |
| 6     |                      |       | Heart, muscle |

* Minor.