Establishment and Characterization of a Novel Human Monocytic Leukemia Cell Line (KP-1) Expressing Scavenger Receptor

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ABSTRACT. A new monocytic leukemia cell line (KP-1) was established from a 2-y-old Japanese girl with acute monocytic leukemia. The KP-1 cells were maintained in suspension culture with a doubling time of 96 h. The cells were positively stained with α-naphtyl butyrate esterase, but not with naphthol AS-D chloroacetate esterase, myeloperoxidase, and periodic acid-Schiff reagent. Cell surface marker analysis revealed that the cells were CD4, CD11a, CD11c, CD13, CD14, CD18, CD33, and HLA-DR positive. Karyotype analysis revealed near diploidy (47 XX) and a translocation t(11;19) was found. When treated with 12-o-tetradecanoylphorbol 13-acetate, KP-1 cells became tightly adherent, showed the enhanced reactivity for α-naphtyl butyrate esterase, and produced several monokines such as IL-1β, tumor necrosis factor-α, and macrophage colony-stimulating factor. Immunoelectron microscopy demonstrated that the human macrophage scavenger receptor was expressed after 12-o-tetradecanoylphorbol-13-acetate treatment, and the cells accumulated a large amount of cholesterol esters in the presence of acetylated LDL. Compared with another human monocytic leukemia cell line, THP-1, KP-1 expressed scavenger receptor and accumulated cholesterol ester more rapidly in the presence of 12-o-tetradecanoyl phorbol-13-acetate and acetylated LDL. Scatchard analysis using ^{125}I-labeled acetylated LDL revealed a typical saturation curve with an apparent k_d of 1.7×10^{-7} M and 3400 binding sites per cell. KP-1 retained the characteristics of monocyte-macrophage lineage cells and will facilitate the *in vitro* studies of the pathologic and physiologic roles of scavenger receptors. (*Pediatr Res* 34: 258–264, 1993)

Abbreviations

TPA, 12-o-tetradecanoylphorbol-13-acetate
CE, cholesterol ester
aLDL, acetylated LDL
TNF, tumor necrosis factor
M-CSF, macrophage colony-stimulating factor
PDGF, platelet-derived growth factor
FBS, fetal bovine serum

Macrophages have been shown to be involved in a number of physiologic processes including presentation of antigens to T and B lymphocytes, resistance to intracellular parasites and bacteria, tumor immunity, and phagocytosis of foreign materials. In addition to these physiologic functions, the pathologic role of macrophages in the atherosclerotic process is highlighted because the foam cells that characterize the early stage of atherosclerotic lesions have been demonstrated to express several macrophage-related phenotypes (1–3). It is generally accepted at present that most of the foam cells in the early stage of atherosclerotic lesions are derived from the circulating monocytes, which differentiate in the subendothelial space to macrophages and are transformed into foam cells in the presence of modified LDL.

Goldstein *et al.* (4) demonstrated that macrophages in culture do not accumulate a large amount of CE if cultured with the native LDL, but if chemically modified LDL such as aLDL is used, the foam cell formation occurs. This result indicates that the uptake of aLDL is mediated by a receptor distinct from the LDL receptor, called the scavenger receptor. Recently, Matsumoto *et al.* (5) succeeded in the molecular cloning of cDNA for the human macrophage scavenger receptor. Then, Freeman *et al.* (6) demonstrated that transfection of cDNA of the scavenger receptor to Chinese hamster ovary cells leads to their conversion to foam-like cells in the presence of modified LDL. Furthermore, several investigators reported that scavenger receptor mRNA and protein have been detected in foam cells in atherosclerotic plaques *in vivo*. (5, 7). These studies indicate that the scavenger receptor pathway plays an important role in the foam cell formation *in vitro* and *in vivo*.

The scavenger receptors are speculated to be involved in other physiologic responses because of their broad binding specificity. In fact, Hampton *et al.* (8) demonstrated that bacterial endotoxin is a ligand of scavenger receptor and hepatic uptake of endotoxin by this receptor plays a significant role in endotoxin clearance. Thus, the scavenger receptor is speculated to play some important, physiologic roles *in vivo*.

Here we report the establishment and characterization of a novel human monocytic leukemia cell line (KP-1) with special reference to the characterization of the scavenger receptor. After TPA treatment, this cell line can be differentiated into mature macrophage-like cells that share many characteristics with normal macrophages.

MATERIALS AND METHODS

Cell cultures. KP-1 was derived from the peripheral blood of a 2-y-old Japanese girl who was refered to Kumamoto University Hospital because of extensive swelling of the gingiva and bleeding from the mucosa of the oral cavity in October 1986. The white

blood cell count of the peripheral blood was $64\,500/\mu L$ at admission and 80% of the cells were monoblasts. The leukemic cells were positive for α-naphtyl butyrate esterase, which was inhibited by NaF. Lysozyme activity in blood was increased to 90 mg/L. Cell surface marker analysis by flow cytometry revealed that the blasts were positive for the MAb that recognize CD13, CD14, and CD33, but T-lymphocyte antigens (CD2, CD3) and B-lymphocyte antigens (CD19, CD20) were negative. The monoblasts were isolated from the peripheral blood by Ficoll-Hypaque density gradient centrifugation and cultured in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% FBS (Gibco) in a humidified 5% CO_2 atmosphere. The medium was changed once a week, and after 2 mo the cells began to proliferate constantly with a doubling time of 96 h. This cell line was stable and no morphologic, cytochemical, and surface marker changes were observed after 10 mo.

Cytologic and cytochemical studies. Morphologic characteristics of the cultured cells were examined by May-Gruenwald-Giemsa staining of a cytocentrifuged preparation of the cells. α-Naphtyl butylate esterase, napthol AS-D chloroacetate esterase, periodic acid-Schiff, and myeloperoxidase staining of the cultured cells were performed by the established methods (9–11).

Cell surface marker. The expression of cell surface antigens were analyzed by using MAb, OKT3 (CD3), OKT4 (CD4), OKT8 (CD8), OKIa1 (HLA-DR) (Ortho Diagnostic, Raritan, NJ), B1 (CD20), B4 (CD19), MY7 (CD13), MY4 (CD14), MY9 (CD33), Mo1 (CD11) (Coulter Immunology, Hialeah, FL), and LFA-1α (CD11a), Leu M5 (CD11c), and LFA-1β (CD18) (Beckton Dickinson Immunocytometry Systems, Mountain View, CA). Cells were incubated with each MAb for 30 min at 4°C, washed with cold PBS, and incubated with FITC-labeled goat antimouse IgG antibody (Ortho Diagnostic) for 30 min at 4°C. Then the cells were washed and subjected to flow cytometry (Spectrum III, Ortho Diagnostic).

Karyotype analysis. Karyotype of the established cell line was examined by a standard trypsin-Giemsa banding.

Lysozyme. Cells (5×10^7 cells/L) were cultured in RPMI 1640 medium supplemented with 10% FBS for 3 d. Then the cells were centrifuged and the supernatant was passed through a 0.45-μm Millipore filter. Lysozyme activity in culture supernatant was assayed by the turbidimetric technique.

Phagocytosis. A 1.0-mL suspension of 2×10^5 KP-1 cells was tested for phagocytic activity by incubation for 60 min at 37°C with a 1.0-μm diameter latex particle (Sekisui Chemical Industry, Osaka, Japan). Furthermore, to evaluate the active phagocytosis via Fc receptor, the latex particle was opsonized by incubation with normal human serum at 37°C for 60 min. The cells were smeared on slide glass and stained with May-Giemsa. Two hundred cells were examined for phagocytic activity.

Immunoelectron microscopy. Generation of anti-human scavenger receptor antibodies and immunostaining were performed by methods reported previously (7). Briefly, antibodies against the scavenger receptor were obtained by s.c. injection of 500 μg of BSA-coupled synthetic peptide emulsified in Freund's complete adjuvant into 2-mo-old male rabbits (Csk:JW/Ina). The synthetic peptides correspond to the sequence in the α-helical coiled coil domain (residue 199–209) and the collagenous domain (325–342) of the type I scavenger receptor. The cell pellet of TPA-treated KP-1 cells was fixed for 10 min in 2% periodate-lysine-paraformaldehyde solution. Then the specimens were incubated with antisera at a dilution of 1:800 to 1:1200. After being washed in PBS, the specimens were covered with goat anti-rabbit Ig conjugated with peroxidase (Amersham, Buckinghamshire, UK). Peroxidase activity was visualized using 3-3'-diaminobenzidine as substrate. Postfixation by osmium tetroxide, dehydration, and embedding were performed by standard procedures. Ultrathin sections were made by an Ultrotome NOVA (LKB, Uppsala, Sweden) and observed through a JEM 2000 EX electron microscope (JEOL, Tokyo, Japan).

Cellular mass of free cholesterol and CE. KP-1 and THP-1 (purchased from American Type Culture Collection, Rockville, MD) were cultured in the presence of TPA for 24 h and then various concentrations of aLDL were loaded. In a preliminary study, maximal accumulation of CE was observed in 20 μg/L TPA for KP-1 and 200 μg/L for THP-1 in the presence of 50 mg/L aLDL. Thereafter, these concentrations of TPA were used for CE accumulation experiments in the two cell lines. The mass of free and esterified cholesterol was quantitated by the method reported by Heider and Boyett (12) with slight modifications. Briefly, aliquots (10 μL) of lipid extract were incubated with 0.4 mL of enzyme mixture at 37°C for 1 h (for free cholesterol) and 2 h (for total cholesterol). Carbowax-6000 in the enzyme mixtures described by Heider and Boyett was changed to 0.01% Triton X-100 and enzymes with 2-fold higher concentrations (cholesterol oxidase: 160 U/L; cholesterol hydrolase: 6000 U/L) were used in this study. Fluorescence intensity was measured with excitation at 320 nm and emission at 407 nm, and the amount of free and total cholesterol was calculated from the standard curves obtained by cholesterol (0–8 nmol) and cholesterol oleate (0–10 nmol) for free and total cholesterol, respectively. The amount of CE was calculated by subtracting the amount of free from total cholesterol. The amount of protein of the samples was determined by the method reported previously (13).

Lipoproteins. Human LDL (density = 1.019–1.063) were prepared by sequential ultracentrifugation from the plasma of healthy volunteers. aLDL was prepared by the method reported by Basu *et al.* (14). aLDL was labeled with ^{125}I (New England Nuclear Corp., Boston, MA) with Iodogen (BioRad Laboratories, Richmond, CA) to a specific radioactivity of 200 cpm/ng. More than 98% of the labeled ligand was trichloroacetic acid–precipitable.

Association of ^{125}I-labeled aLDL. KP-1 was seeded in plastic culture wells (Corning, Iwaki glass, Tokyo, Japan; 16 mm in diameter) at a concentration of 1.0×10^{10} cells/L in 1.0 mL of RPMI 1640 medium supplemented with 10% FBS plus 20 μg/L TPA and cultured for 24 h in a 5% humidified CO_2 atmosphere at 37°C. The monolayers were washed three times with 1.0 mL of Eagle's minimum essential medium (Gibco) containing 3% BSA (Fraction V, Sigma Chemical Co., St. Louis, MO) buffered with 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Sigma) to pH 7.4 (medium A). Each well was incubated at 37°C with varying concentrations of ^{125}I-labeled aLDL in 0.5 mL of medium A. After 4 h of incubation, adherent cells were washed three times with 1.0 mL of medium A and five times with PBS. Cells were dissolved for 3 h at 37°C in 1.0 mL of 0.1 N NaOH to determine the cell-associated radioactivity. Nonspecific cellular association was determined by parallel culture with 1.0 g/L of cold ligand. Specific cellular association was determined by subtracting nonspecific from total cell association.

Monokine production. The concentrations of TNF-α, IL-1α, IL-1β, and M-CSF were determined by specific immunoassay in the culture media from *1*) unstimulated, *2*) TPA-stimulated, and *3*) aLDL-loaded KP-1 cells. The enzyme immunoassay kits for TNF-α, IL-1α, and IL-1β were purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan).

The method for enzyme immunoassay for M-CSF was described previously (15). The unstimulated cells were cultured at a concentration of 1.0×10^9 cells/L in RPMI 1640 medium supplemented with 10% FBS and TPA-stimulated cells were cultured in the same medium in the presence of 20 μg/L TPA for 48 h. The aLDL-loaded cells were cultured with 20 μg/L TPA for the first 24 h and then cultured in the presence of 50 μg/L of aLDL for an additional 24 h. The culture supernatants were centrifuged at 1200 rpm for 10 min, passed through a 0.45-μm Millipore filter, and subjected to each immunoassay.

RESULTS

Cytologic and cytochemical characteristics of KP-1 cells. KP-1 cells were homogenous suspension cells with an average doubling time of 96 h. The cells were round and had membrane ruffles or pseudopods. They had a round nucleus with fine chromatin distribution and two to four nucleoli. The basophilic cytoplasm contained some granules. After treatment by 20 μg/L TPA, this cell line became tightly adherent to plastic surfaces within 24 h. These cells were flat, had a reduced nuclear/cytoplasmic ratio, and had multiple projections on their surface membranes. There was a marked increase of vacuoles in cytoplasm and the granules were less prominent (Fig. 1*A* and *B*). Mitotic figures, which were frequently observed in untreated KP-1 cells, were never seen after TPA treatment. Cytochemically, more than 90% of TPA-stimulated cells showed intense cytoplasmic staining for α-naphtyl butyrate esterase, which was completely inhibited by NaF, whereas about 60% of the nonstimulated cells were less intensely stained for this enzyme. Naphthol AS-D chloroacetate esterase, myeloperoxidase, and periodic acid-Schiff staining were negative before and after TPA treatment.

Foam cell formation. When KP-1 cells were preincubated with 20 μg/L of TPA for 12 h and then cultured in the presence of 20–100 mg/L aLDL for an additional 24 h, the cells were transformed into typical foam cells that contained many lipid droplets (Fig. 1*C*). The size and number of lipid droplets increased in parallel with the concentration of aLDL added. On the other hand, without TPA stimulation the cells were not transformed into foam cells in the presence of aLDL.

Surface marker analysis. The results of the surface marker analysis are summarized in Table 1. The KP-1 cells expressed the antigens that were recognized by OKT4 (CD4), MY7 (CD13), MY4 (CD14), MY9 (CD33), LFA-1α (CD11a), Leu M5 (CD11c), LFA-1β (CD18), and OKIa1 (HLA-DR). This cell line did not react with the MAb that recognize T (CD 2, CD 3, CD 8) and B (Cd 19, CD 20) lymphocytes, showing that the binding of the cell line to the above-mentioned MAb was not due to nonspecific binding to Fc receptor (Table 1).

Karyotype analysis. Chromosome analysis of 10 cells showed near diploidy (modal chromosome number 47, range 46–48). A translocation t(11;19) and other abnormalities, 1p⁻, 10p⁺, 16q⁺ and 19 p⁺, were commonly observed (Fig. 2).

Lysozyme production. When KP-1 cells (5 × 10⁷ cells/L) were cultured in RPMI 1640 medium supplemented with 10% FBS for 3 d, lysozyme activity in the culture supernatant was 2.0 mg/L. The detection limit of this assay was 0.05 mg/L and the lysozyme activity in fresh culture medium was undetectable.

Phagocytosis. The percent phagocytosis of unopsonized particle in unstimulated and TPA-stimulated (20 μg/L, for 24 h) cells was 3 and 12%, respectively. When opsonized particle was used, the percent phagocytosis in unstimulated and stimulated cells increased to 13 and 46%, respectively.

Expression of scavenger receptor in KP-1 cells. Immunoelectron microscopy showed positive staining on the cell surface membrane and occasionally on the membrane of the endocytic vesicles in the TPA-stimulated KP-1 cells, whereas the unstimulated cells showed no positive reactions (Fig. 3).

Endocytic uptake of aLDL by KP-1 cells. When KP-1 cells were stimulated by 20 μg/L TPA for 24 h, cell-associated ¹²⁵I-aLDL exhibited a typical saturation curve against the ligand concentration (Fig. 4). Furthermore, the addition of excess unlabeled ligand decreased the amount of cell-associated ¹²⁵I-aLDL by 80%. With the Scatchard analysis, the specific cell-associated ¹²⁵I-aLDL gave an apparent k_d for uptake of 1.7×10^{-7} M and 3400 binding sites per cell. These results demonstrated the presence of specific receptors for aLDL on the cell surface of the KP-1 cells.

Accumulation of CE in KP-1 cells loaded with aLDL and native LDL. When KP-1 cells were stimulated by 20 μg/L TPA for 24 h and then loaded with aLDL for an additional 24 h, the amount of accumulated CE increased in parallel with the concentration of aLDL loaded. Approximately nine times the baseline CE was accumulated when 100 mg/L aLDL were loaded. When native LDL was used, only a slight increase of CE accumulation was observed at 25 and 50 mg/L loading (Table 2). This result indicated that the stimulated KP-1 cells accumulated a large amount of CE via scavenger receptor, but not via LDL receptor. The unstimulated cells did not accumulate a significant amount of CE in the presence of aLDL (data not shown).

Comparison of time course CE accumulation between KP-1 and THP-1 cells. KP-1 and THP-1 cells were differentiated by 24-h culture in RPMI 1640 medium supplemented with 10% FBS in the presence of 20 μg/L TPA for KP-1 cells and 200 μg/L TPA for THP-1 cells. Then 50 mg/L aLDL was loaded and
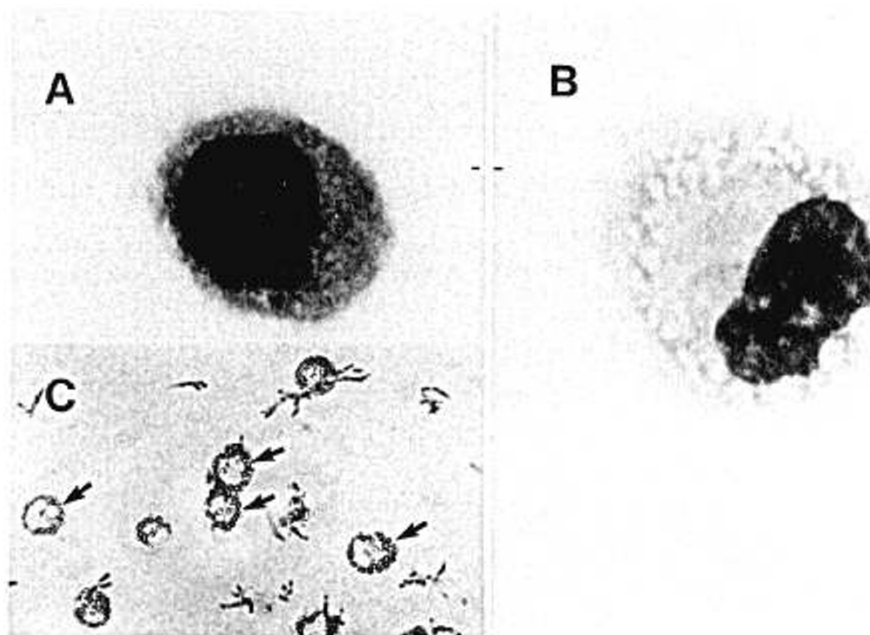

Fig. 1. Light microscopic photographs of unstimulated (*A*) and TPA-stimulated (*B*) (20 μg/L, for 24 h) KP-1 cells stained with May-Giemsa staining (×1000) and foam cells stained with Sudan III (*C*) (×400). *Arrows* indicate the typical foam cells.

Table 1. *Reactivity of KP-1 with MAb*

Antigen	Positive cell (%)
CD 2	(−)*
CD 3	(−)
CD 4	80.4
CD 8	(−)
CD 11a	35.9
CD 11c	91.2
CD 13	92.0
CD 14	84.1
CD 18	66.2
CD 19	(−)
CD 20	(−)
CD 33	76.6
HLA-DR	81.5

* Positive cells were less than 5%.

time course of CE accumulation in both cell lines was obtained. In KP-1, a significant amount of CE accumulation was observed in 24 h and the amount rapidly increased in parallel with the time course and reached maximum in 72 h after aLDL loading. In contrast, THP-1 accumulated CE very slowly for the first 48 h and the amount increased rapidly after 72 h. The difference of maximal amount of CE accumulated was not significant between the two cell lines (Fig. 5).

Monokine production. TNF-α, IL-1α, IL-1β, and M-CSF were below detection limit in culture supernatant from unstimulated KP-1 cells. When KP-1 cells were stimulated by 20 μg/L TPA, the concentration of TNF-α, IL-1β, and M-CSF increased to 11.3 ng/L, 25.0 ng/L, and 8600 × 10³ U/L, respectively. After KP-1 cells were transformed into foam cells, the concentration of TNF-α, IL-1β, and M-CSF further increased to 25.2 ng/L, 49.0 ng/L, and 9800 × 10³ U/L, respectively. IL-1α was not detected in any samples (Table 3).

DISCUSSION

The present report described the establishment and characterization of a novel human monocytic leukemia cell line, KP-1, which was derived from a 2-y-old Japanese girl with acute monocytic leukemia. This cell line showed the following characteristics: *1)* the presence of α-naphtyl butylate esterase, which was inhibited by NaF; *2)* lysozyme production; *3)* phagocyte activity of the latex particle; *4)* expression of CD13, CD14, CD33, CD11a, CD11c, CD18, and HLA-DR antigens on the cell surface; *5)* production of several monokines, TNF-α, IL-1β, and M-CSF, after TPA treatment; *6)* induction of scavenger receptor expression after TPA treatment; and *7)* accumulation of a large amount of CE and transformation into foam cells in the presence of TPA and aLDL. These results demonstrated that this cell line represents a monocyte-macrophage lineage cell and can be differentiated into mature macrophage-like cells after TPA treatment.

Macrophage scavenger receptors are implicated in the pathologic deposition of cholesterol in arterial walls. The expression of scavenger receptor in the present cell line was demonstrated by immunostaining using the antibodies against human macrophage scavenger receptor, but its expression was limited to the differentiated cells. This result was compatible with the observation that only the differentiated cells accumulated a large amount of CE in the presence of aLDL, confirming that the uptake of aLDL was made via scavenger receptor. The immunostaining demonstrated that cell surface membrane and occasionally membrane of the endocytic vesicles were positively stained, indicating that the uptake was made by invagination of cell surface membrane and subsequent endocytic vesicle formation. Scatchard analysis using ^{125}I-aLDL revealed that the apparent k_d for uptake was 1.7×10^{-7} M and the number of binding sites per cell was 3400. These values were similar to those of another monocytic leukemia cell line, THP-1, which also expresses the scavenger receptor after differentiation and accumulates a large amount of CE in the presence of aLDL (16).

Among several human cell lines representing macrophage characteristics, THP-1 and U-937 were often used as an *in vitro* model for foam cells in atherosclerotic lesions (17–21). THP-1 and U-937 were established from a patient with acute monocytic leukemia and one with histiocytic lymphoma, respectively (22, 23). However, there are some differences in the pathway and the time course of the accumulation of CE between the cell lines. U-937 accumulates CE via both the scavenger and the LDL receptor pathway (24), whereas THP-1, KP-1, and monocyte-derived
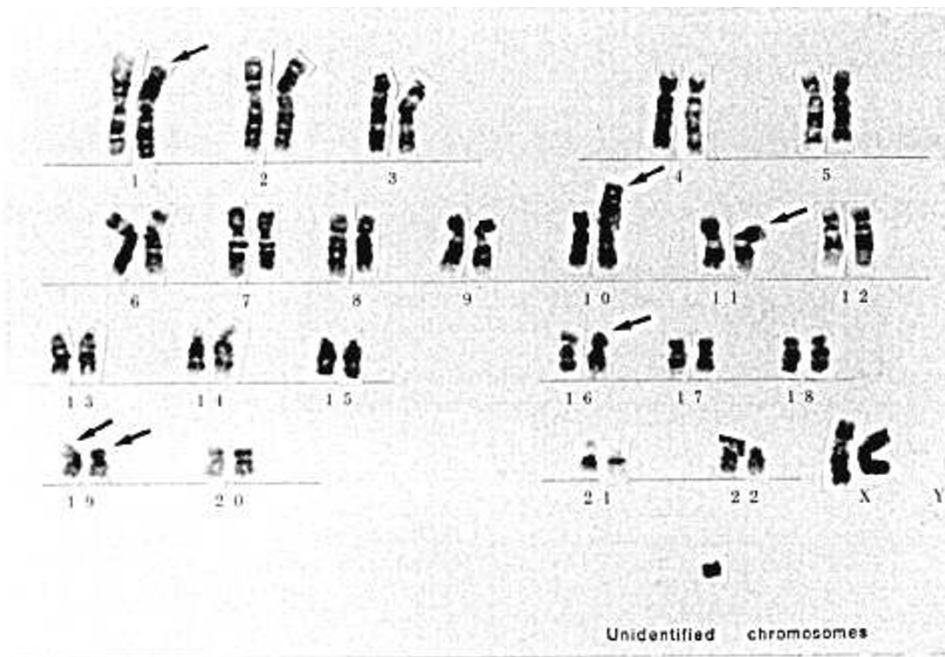

Fig. 2. The representative karyotype of KP-1, showing 47 XX, t(11q⁺;19p⁻), 1p⁻, 10p⁺, 16q⁺, 19p⁺, + mar. *Arrows* indicate the translocated chromosomes.
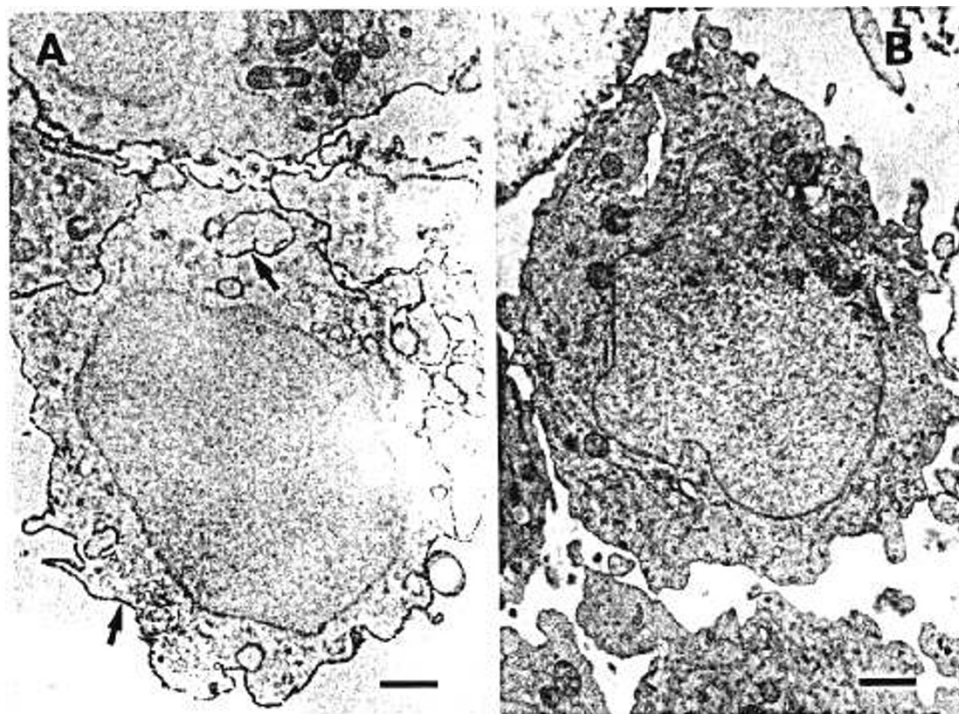
Fig. 3. Electron microscopic photograph of KP-1 immunostained with the antibodies against human macrophage scavenger receptor. Positive staining (*arrows*) is found in the cell surface membrane and the membrane of endocytic vesicles in TPA-stimulated cells (*A*, ×8800). In contrast, no positive staining is found in unstimulated cells (*B*, ×9800).
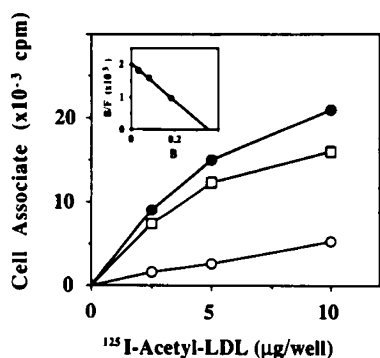

Fig. 4. Cell-associated ^{125}I-aLDL as a function of ligand concentrations in KP-1. Cells were incubated with ^{125}I-aLDL in the presence (nonspecific cell-association, O) or absence (total cell association, ●) of 1 mg/mL unlabeled aLDL. □ denotes the specific uptake and Scatchard analysis is shown in the *inset*.

Table 2. *Cellular mass quantitation of total cholesterol, free cholesterol, and cholesterol esters in KP-1 loaded with aLDL and native LDL**

Treatment (μg/well)	TC (nmol/mg cell protein)	FC (nmol/ cell protein)	CE (nmol/mg cell protein)	p value
aLDL				
0	94.16 ± 9.61	85.51 ± 9.52	8.65 ± 1.47	
25	147.30 ± 7.39	106.76 ± 3.06	40.54 ± 4.33	<0.001
50	171.36 ± 12.14	111.47 ± 8.13	60.16 ± 4.33	<0.001
100	177.24 ± 7.15	100.10 ± 4.35	77.14 ± 2.94	<0.001
LDL				
0	94.86 ± 2.57	85.93 ± 2.90	8.93 ± 0.78	
25	100.58 ± 6.24	89.10 ± 5.93	11.48 ± 0.32	<0.05
50	105.13 ± 3.35	87.61 ± 3.37	17.51 ± 3.02	<0.05
100	89.04 ± 3.47	79.09 ± 2.67	12.95 ± 3.99	NS

* Results are expressed as mean ± SEM of triplicate assays and the increase in the accumulated amount of CE was statistically evaluated by using the paired *t* test. TC, total cholesterol; FC, free cholesterol; and CE, cholesterol esters.

macrophage accumulate CE only from the scavenger receptor pathway after differentiation (17). Compared with widely used THP-1, KP-1 showed a very distinct time course of CE accumulation. As shown in Figure 5, KP-1 accumulated a large amount of CE much more rapidly than THP-1. This result indicated that the functional expression of the scavenger receptor was induced more rapidly in KP-1 than in THP-1. In fact, as Via *et al.* (25) reported that the scavenger receptor expression of THP-1 cells reaches optimal levels after 72 h of TPA treatment and the delayed expression of the scavenger receptor is one of the possible explanations for the delayed accumulation of CE in THP-1 cells. These characteristics of KP-1 may be practically important and may facilitate the *in vitro* studies of scavenger receptor function and the mechanism of foam cell formation.

Another characteristic of KP-1 cells is that this cell line retains the capacity to produce several monokines, TNF-α, IL-1β, and M-CSF. This cell line produced these monokines after differentiation by TPA. It is of interest to note that the monokine

production was further augmented along with the transformation of this cell line into foam cells (Table 3). TNF-α and IL-1β have been suggested as the potential enhancer of the atherosclerotic lesions. These monokines stimulate PDGF production from endothelial cells and smooth muscle cells (26, 27). PDGF from endothelial cells may induce migration of smooth muscle cells into the atherosclerotic lesion and PDGF production from smooth muscle cell may stimulate its own proliferation by autocrine mechanism. The accumulation of smooth muscle cells leads the early stage of atherosclerotic lesions to more advanced plaques. Furthermore, several investigators have reported that both monokines induce expression of leukocyte adhesion molecules on the surface of endothelial cells (28, 29). Therefore, these monokines will enhance the infiltration of leukocytes into the atherosclerotic lesion, which will accelerate the atherosclerotic process. With respect to M-CSF, this monokine can also be atherogenic by enhancing the expression of scavenger receptor
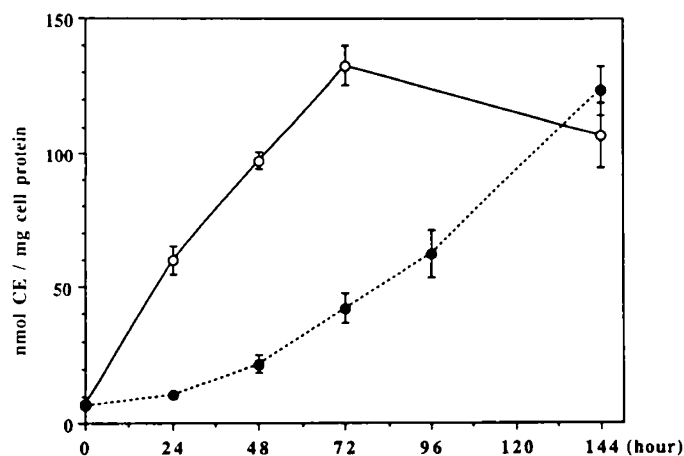
Fig. 5. Comparison of time course for CE accumulation between THP-1 and KP-1. THP-1 (●) and KP-1 (○) were incubated for 24 h with 200 and 20 μg/L TPA, respectively. Then 50 mg/L aLDL was loaded and the accumulated amount of cholesterol ester was quantitated at the time point indicated for an additional 24 h. Each value is expressed as mean ± SEM of triplicate assays.

Table 3. *Effect of TPA and aLDL loading on monokine production from KP-1**

| | Monokine | | |
Treatment	TNF-α (ng/L)	IL-1β (ng/L)	M-CSF (U × 10⁻³/L)
No treatment	UD	UD	UD
TPA	11.3	25.0	8800
TPA + aLDL	25.2	49.0	9900

* Results are expressed as mean of duplicate assays. Detection limits of TNF-α, IL-1β, and M-CSF were 2 ng/L, 4 ng/L, and 50×10^3 U/L, respectively. UD, undetectable.

and the uptake of cholesterol (30, 31). Therefore, these lines of evidence suggest that foam cell formation could further accelerate the atherosclerotic process via augmented production of the monokines.

The expression of macrophage functions is closely related to the differentiation stage. Complement production, cytotoxic activity, phagocytic activity, chemotactic responses, and the production of mediators of inflammation have all been shown to be maximally expressed at specific stages of differentiation (32, 34). Therefore, it would be necessary to prepare homogenous macrophages in the same differentiation stage to investigate their functions. In this sense, the present cell line, being homogenous in the differentiation stage and expressing scavenger receptors by short-term incubation with TPA will be useful and facilitate *in vitro* studies of the pathologic and physiologic functions of macrophages via scavenger receptors.

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Announcement

Research on Hair Testing for Drugs of Abuse

The National Institute on Drug Abuse has an interest in research on the utility of hair as a specimen for the detection of drugs of abuse. The goals of this program are to encourage systematic research on the use of hair testing to detect drugs of abuse in an accurate and reliable manner and to develop the necessary procedures and safeguards. Specific areas of interest are pharmacologic studies, analytical studies, and cost/benefit ratio analyses. Further information may be obtained from Rao S. Rapaka, Ph.D., or M. Beth Grigson Babecki, M.A., Division of Basic Research, National Institute on Drug Abuse, 5600 Fishers Lane, Room 10A-31, Rockville, MD 20857, (301) 443-6975. (Program announcement no. PA-92-18, November 1, 1991–November 1, 1994)