An In Vitro Coculture Model of Transmigrant Monocytes and Foam Cell Formation

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Abstract—To analyze in vitro the migration of monocytes to the subendothelial space, their differentiation into macrophages, and the subsequent formation of foam cells in vitro, we have developed a 2-coculture system with rabbit aortic endothelial cells (AECs), aortic smooth muscle cells (SMCs), and a mixture of matrix proteins on polyethylene filters in chemotaxis chambers. AECs were seeded on a mixture of type I and IV collagen with or without various types of serum lipoproteins (method 1) or on matrix proteins secreted by SMCs (method 2). In these coculture systems, rabbit AECs can maintain a well-preserved monolayer for up to 2 weeks. When human CD14-positive monocytes were added to the upper medium of the system, with monocyte chemotactic protein-1 treatment, monocytes transmigrated within 24 hours and were retained for up to 7 days, whereas without MCP-1 treatment, <30% of monocytes transmigrated. On day 1, transmigrant monocytes were negative for immunostaining of type I and II macrophage scavenger receptors but by day 3, became positive for scavenger receptors as well as other macrophage markers. When oxidized low density lipoprotein was added to the matrix layer of the method I coculture, on day 4 transmigrant cells exhibited lipid deposit droplets, and by day 7, they had the appearance of typical foam cells. Some of the transmigrant cells recovered in the lower medium on day 7 also appeared to be foam cells, indicating foam cell motility and escape from the coculture layer through the filter. In summary, this coculture system is a useful in vitro tool to dissect the cellular and molecular events that make up the process of foam cell formation. (Arteriosclerosis and Thrombosis. 1999;19;2330-2339.)

Key Words: atherosclerosis ■ chemoattractants ■ macrophages ■ foam cells ■ oxidized LDLs

A key cellular component of atherosclerosis is the lipid-laden macrophage termed the foam cell. In animal models of atherosclerosis and in human subjects with hypercholesterolemia, increased levels of plasma cholesterol cause the migration of monocytes into the subendothelial space of the arterial wall, where they differentiate into macrophages and subsequently generate foam cells.1–4 However, many aspects of the cellular mechanisms by which foam cells are formed remain unknown. Nevertheless, several previous attempts have opened a window on approaches to this cellular process in vitro by using cultured aortic endothelial cells (AECs) and smooth muscle cells (SMCs).5–11 Monocyte migration into the subendothelial space in a coculture of adult human AECs and SMCs was previously reported,9 and its use was suggested for study of the cellular processes of atherogenesis. One of the major difficulties of this system, unfortunately, is the limited stability of the AEC monolayer, which can be easily disturbed by underlying matrix components and other factors, including growth factors and cytokines.12,13 This presents an obstacle: for example, in the rabbit model on a high-cholesterol diet, it takes at least 1 to 2 weeks for sufficient lipid to accumulate in the vascular wall and an additional 1 to 2 weeks for foam cells to appear.14,15 Thus, for an adequate analysis of foam cell formation, the AEC monolayer in the in vitro coculture system needs to be stable for at least 2 weeks.

In an effort to establish a more stable system, we selected rabbit AECs and SMCs as the components of the coculture. Rabbit AECs are derived from the artery, which is prone to develop atherosclerotic lesions, and lipid metabolism and atherogenesis in animal species have been studied intensively.14–18 We here report an improved method for primary culture of rabbit AECs and the effect of matrix proteins on the establishment of a longer-lasting culture, since type I collagen
or MATRIGEL cannot support the AEC monolayer for 2 weeks. We used 2 different methods. Either a mixture of type I and type IV collagen (method I) or a mixture of matrix proteins secreted from rabbit SMCs (method II) was used to stabilize the continuity of the endothelial monolayer.

Using these 2 methods, we studied the effect of monocyte chemotactic protein-1 on monocyte transmigration and foam cell formation. The transmigration of fluorescently labeled monocytes was quantitatively analyzed by 3-dimensional analysis with confocal laser microscopy. Each step of this transmigration was also studied with electron microscopy and immunohistochemical analysis. Finally, we report the formation of foam cells in these systems with an appearance similar to those found in human atherosclerotic lesions.

Methods

Reagents
The reagents and their suppliers are as follows: Dulbecco’s modified Eagle’s medium (DMEM), M199, trypsin-EDTA, penicillin-streptomycin, and other tissue culture materials from Gibco BRL, Life Technologies Inc; PBS from the Takara Co Ltd; endothelial growth medium-2, the culture medium for ECs, including EC growth supplements, from Clonetics Corp; collagenase type 2 from Worthington Biochemical Corp; 96-well and 24-well tissue-culture plates from Becton Dickinson Labware; 60-mm and 100-mm collagen type I–coated dishes from the Iwaki Co Ltd; the insert well, Chemotaxis-cell (filter surface area 0.48 cm², pore size 3 μm in diameter) from the Kurabo Co Ltd; living cell fluorescent staining kit PKH2 (FITC-like fluorescent pigment) and PKH26 (rhodamine-like fluorescent pigment) from Synaxis Cell Science, Inc; collagen type I and IV solution from the Koken Co Ltd; Microbead-labeled anti-CD14 antibody, MidiMACS, and single-use, type BS separation columns from Miltenyi Biotec GmbH; and recombiant human MCP-1 from Pepro Tech EC Ltd.

Lipoproteins
Lipoproteins were separated from the plasma of a normal healthy volunteer, and LDLs were oxidized by a 16-hour incubation in the presence of 10 μmol/L CuSO₄ in RPMI.

Cell Culture
Rabbit AECs
A male, Japanese White rabbit (1.0 to 1.5 kg) was anesthetized by intravenous injection of pentobarbital. The midline of the abdomen was incised, and all of the aorta was taken from the abdominal aorta at the bifurcation by using a heparin-flushed syringe. The thoracic portion of the aorta was dissected out. The connective tissue and fat were removed, and a segment of aorta was dipped in DMEM (containing 5% FCS). After being washed with serum-free DMEM, the distal end of the fragment was fastened with a thread, and the proximal end was bound with the syringe joint. Collagenase type 2 solution (400 U/mL, dissolved in serum-free DMEM) was poured into the aorta, and the segment was incubated for 30 minutes at 37°C. After collagenase digestion, ECs were removed from the aorta by flushing with 25 mL of DMEM (with 5% FCS) twice. Cells were collected by centrifugation at 70g for 5 minutes and were resuspended in 50 mL of DMEM supplemented with 5% FCS, 100 μg/mL penicillin, and 100 μg/mL streptomycin. Cells were dispersed in ten 96-well plates at 50 μL per well. To remove contaminated SMCs, after a 4-hour incubation at 37°C, the wells of these plates were washed twice with 300 μL of PBS in a 12-channel 96-well plate-washing machine (Flexiwash I, Towa Lab Co). Adherent cells, almost all of which were ECs, were cultured for ~7 days with EGM-2 and 2% rabbit serum at 37°C. When the ECs reached 80% confluence, they were passed into a 60-mm collagen type I–coated culture dish, with the use of 0.05% trypsin in 0.33 mmol/L tetrasiomd EDTA. Rabbit AECs from 5 wells were cultivated in one 60-mm dish for 7 days without changing the medium. The cells were checked by morphology, Di acetyl LDL uptake activity, and immunocytostaining with an anti–von Willebrand factor antibody. Rabbit AECs from the subculture of 3 or 4 passages were used in this study without freezing and thawing.

Rabbit Aortic SMCs
Rabbit SMCs were isolated from the aortic media by an explant technique modified from that of Ross. After removal of the ECs, the segments were cut open and the media separated by means of a thin pinccette. During these procedures, the segments were dipped in DMEM with 5% FCS. The isolated medial fragments were minced into small pieces, and these were scattered on the dish and dried for ~2 to 5 minutes. DMEM (with 5% FCS) warmed at 37°C was poured slowly into the dish. The fragments were cultivated for 7 days at 37°C in 95% air/5% CO₂. Migrant SMCs were passed by using 0.05% trypsin in 0.53 mmol/L tetrasiomd EDTA. SMCs were defined by an immunocytostaining procedure with anti-rabbit SM myosin chain antibody (SM1, Yamasa Co Ltd). Rabbit SMCs from the subculture of 3 to 7 passages were used in this study without freezing and thawing.

Human Peripheral Blood Monocytes
Monocytes were isolated from the whole blood of a healthy donor. Mononuclear cells were enriched by centrifugation with a Ficoll-Paque Plus (Pharmacia Biotech) gradient. Monocytes were isolated by a magnetic cell-sorting apparatus with the use of microbead-labeled anti-human CD14 antibody (MACS system). More than 95% of the purified cells were positive for CD14. Most exhibited the morphology of human monocytes and adhered immediately (within 5 minutes) to glass plates.

Construction of a Vascular Model
Method 1
Reagents for the matrix layer, ie, collagen type I solution (0.5%, Koken), collagen type IV solution (0.3%, Koken), 10× conditioned medium 199, and reconstruction buffer (0.05 mol/L NaOH,
200 mmol/L HEPES, and 0.26 mol/L NaHCO₃) were mixed in the following volumetric proportions at 4°C: 32:48:10:10, respectively. The mixture was laid on the Chemotaxicell filter, and incubated at 37°C for 1 hour to complete gel formation. Rabbit AECs (2 × 10⁵) labeled with PKH-26 were seeded on the gel. One week later, rabbit AECs became confluent and were ready for the following experiments.

Method 2
SMCs (10⁶) were laid on the filter and cultivated with EGM-2 (with 2% rabbit plasma) for 7 days. After a sufficient amount of matrix proteins had been secreted from the SMCs, rabbit AECs labeled with PKH26 were seeded on the SMCs and their secreted matrix proteins and proteoglycans. One week later, they were used for experiments.

Quantitative Analysis of Monocyte Transmigration With or Without MCP-1 Treatment
After the rabbit AEC monolayer became confluent, both the upper and lower media were changed to a medium containing recombinant human MCP-1 so that cell surface heparan sulfate could sequester chemokines. A preliminary study had indicated that monocyte transmigration was stably enhanced at concentrations of 10 to 100 nmol/L, and for these experiments, a concentration of 30 nmol/L MCP-1 was used. After a 4-hour incubation, the upper medium was removed, and the well was washed twice with PBS. Monocytes (10⁵ CD14-positive cells) were then added to a single chamber. To maintain the MCP-1 gradient, the lower medium was changed to a fresh medium containing 30 nmol/L MCP-1, and the coculture systems were incubated for various periods of time at 37°C. At days 1, 4, and 7, the endothelial surface of each filter was washed independently with 400 μL of PBS 3 times and with 1 mL of PBS for the outside of the chamber twice. The housing was then cut off, and a quantitative assay with confocal microscopy (LEICA TCS SP, Leica Microsystems) was performed. Also, the number of cells in the matrix layer and the number of cells attached to the ECs were counted by using confocal microscopy. For each count, the recovered upper medium and 1.2 mL of the PBS used for washing the endothelial layer were collected together to count the total number of living, floating monocytes.

Histopathological Analysis
Silver Nitrate Staining and Immunocytochemistry
After the experiments, the cell layers on the filters were fixed with 4% paraformaldehyde fixative for 15 minutes at 4°C and washed with PBS. To confirm formation of the AEC monolayer, the samples were stained with silver nitrate by using the method described by Furie et al. Anti-catenin antibody was kindly provided by Drs Akira Nagafuchi and Sho-ichiro Tsukita. Anti–PECAM-1 (CD31) antibody was kindly provided by Drs Michitaka Masuda and Keigi Fujiwara. Anti–VE-cadherin antibody (C-19) was supplied by Santa Cruz Biotechnology Inc. These antibodies were used as markers for tight junctions and adherence junctions in the cells. For the study of differentiation of monocytes into macrophages, the
samples were embedded in a tissue-embedding compound and frozen in LN₂ for subsequent preparation of frozen sections. After endogenous peroxidase activity was blocked, the sections were incubated with MH-1, an anti-human type I and II scavenger receptor monoclonal antibody, and KP-1 (CD68, Dako) for 1 hour. The anti-macrophage cell-surface marker antibody AM-3K (at the time of this writing, the antigen for the AM-3K antibody has yet to be characterized) was also used for immunohistochemical analysis. The sections were then reacted with anti-mouse Ig [F(ab')₂] conjugated with peroxidase (Amersham) for 1 hour. To visualize peroxidase activity, 3,3-diaminobenzidine was used as the substrate. Counterstaining was done with hematoxylin. As controls, sections were incubated with nonimmunized mouse serum or PBS instead of primary antibody and then processed as described above.

Electron Microscopy
For scanning electron microscopic observation, the cell layers on the filters were fixed with 1.2% glutaraldehyde for 4 hours, followed by 1% OsO₄ for 1 hour. The specimens were dehydrated with a graded ethanol series and isoamyl acetate. They were desiccated by the critical-point method using CO₂ and were coated with metal in an ion coater (type IB-3, EIKO Engineering Co). The specimens were observed in a Hitachi S-800 scanning electron microscope (Hitachi) at an accelerating potential of 20 kV. For transmission electron microscopic observation, the cell layers on filters were fixed with 1.2% glutaraldehyde for 60 minutes, washed with 0.1 mol/L cacodylate buffer (pH 7.4), and further fixed with 1% OsO₄, as described above. Then the samples were again dehydrated through a graded series of ethanol solutions. After the filters were removed from the cell layers, the latter were passed through propylene oxide and embedded in epoxy resin. Ultrathin sections were cut with an ultramicrotome (MT-7000, Research and Manufacturing) and examined with an electron microscope (H-7500, Hitachi) after being counterstained with uranyl acetate and lead citrate.

Statistics
Results are given as the mean±SEM. Statistical comparisons were made by the 2-tailed Student’s paired t test. Results were considered significant at P<0.01.

Results
Formation of the Endothelial Monolayer on the Collagen Layer
Figure 1 indicates the hematoxylin staining of the coculture systems used in this article. Rabbit AECs were cultivated for 7 days on either a mixture of type I and type IV collagen (method I, Figure 1a) or matrix proteins secreted from rabbit SMCs (method 2, Figure 1b). We optimized rabbit AEC growth with a mixture of 0.5% type I solution and 0.3% type IV collagen solution in a range of ratios between 1:9 and 9:1 (vol/vol), with the ratio of 4:6 (vol/vol) of type I to type IV, which yields the ratio of 9:10 (wt/wt), providing the best attachment and monolayer growth. At this content (method I), rabbit AECs can maintain a fully functional monolayer with continuous tight junctions for up to 14 days, as judged by silver nitrate staining (Figure 2a and 2d) or immunohistochemistry with anti-catenin (Figure 2b and 2e) and anti-VE-cadherin antibodies (Figure 2c and 2f). The rabbit AEC monolayer on SMCs (method II) also is able to maintain a well-preserved monolayer for up to 14 days.

Transmigration of Monocytes
As shown in Figures 3a and 3b, rabbit AECs were labeled with PKH26 (red) and human monocytes with PKH2 (green). After the sample was fixed with 4% paraformaldehyde solution, the coculture system on the microporous membranes was carefully separated from the plastic housing and placed on glass slides. The vertical sectioning observation of samples by confocal microscopy clearly indicates the position of the monocytes. The monocytes were attached to the AEC layer (Figure 3a), and the transmigrant monocytes (Figure 3b) are clearly identifiable.
For quantitative analysis, the microporous filter was divided into 28 separate areas as indicated in the inset on the left in Figure 3. The center of each area was scanned vertically by using a computer-assisted scanning table, and the number of monocytes either attached to rabbit AECs or transmigrated into the matrix layer was counted in the 90×125-μm² area.

For the initial attempts to analyze monocyte transmigration, the confluent monolayer of rabbit AECs in method 1 was pretreated for 4 hours by the addition of 30 nmol/L MCP-1. The coculture systems (method 1) were then incubated with fresh medium with 30 nmol/L MCP-1. They were coincubated with 30 nmol/L MCP-1 for 4 hours, and 10⁵/chamber of monocytes were added. At the same time, the lower chambers were changed to fresh AEC medium containing 30 nmol/L MCP-1. Then, 10⁵ CD14-positive monocytes were added to the upper medium, and the numbers of monocytes in the collagen layer and of floating monocytes were counted. Figure 4 indicates the time course of monocyte migration in the method 1 culture system. After 24 hours of incubation with the monocytes, >60% of the monocytes were detected within the matrix layer, and this percentage did not change for up to 7 days. However, in the absence of MCP-1 treatment, <30% of the monocytes were detected in the matrix layer throughout the 7 days.

Ultrastructure of the Transmigrant Monocytes
Examination by scanning electron microscopy of the method 1 coculture system treated with MCP-1 revealed many of the features of monocyte transmigration previously reported in experimental animal models (Figure 5). Monocytes attached to the rabbit AECs (Figure 5a) displayed a variety of plasma membrane configurations, including “ruffles” (lamellipodia), filopodia, microvilli, and pseudopodia, suggesting that they were already activated. Transmigrating monocytes exhibited a well-developed, veil-like structure (Figures 5b and 5c). Some monocytes also exhibited structures known as lamellipodia (Figure 5b, arrowhead) and uropodia (Figure 5b, arrow). The position of the endothelial junction and its relationship to the transmigrating monocytes could not be identified in these pictures, and further studies will be needed. During fixation, the AEC layer at times dissociates from the collagen layer, and this may be ascertained by the position of micropores on the filter membrane (indicated as P in Figure 5d). The monocyte in Figure 5d is moving through the basement membrane of the AEC layer (bEC). Below the AEC layer, the fixed matrix layer can be seen in the form of entangled fibers. During migration, the monocytes appeared to be digesting these fibers (Figure 5e).³⁰

An increase in the number of transmigrant monocytes was also detected in the matrix layer of method 2. To confirm monocyte differentiation into macrophages, we characterized these transmigrant cells by immunostaining for macrophage-specific cell surface markers, including the types I and II macrophage scavenger receptors,³¹ CD68 and AM-3K antigen. On day 1, monocytes attached to rabbit AECs (Figures 6a through 6c, arrows), or monocytes at the site of rabbit AECs (Figures 6a, arrowheads) were positive for CD68 (Figure 6a) but negative for immunostaining of type I and II macrophage scavenger receptors (Figure 6b) or AM-3K antigen (Figure 6c). These results are consistent with the reported pattern of cell surface markers of typical human monocytes.³¹–³³ On day 3, they became positive for scavenger receptors as well as for AM-3K antigen (Figures 6d through 6f, arrowheads), indicating that they had already differentiated into macrophage-like cells at this point. Monocytes transmigrated into the matrix layer of method 1 also exhibited positive staining for type I and II scavenger receptors and AM-3K antigen.

Foam Cell Formation in the Coculture Systems
Figure 7 indicates the morphological changes that occurred during transmigration. In method 1, oxidized LDL was added to the collagen solution at 20 μg/mL at 4°C before it was applied to the microporous filter. After polymerization of the collagen gel, rabbit AECs and then monocytes were laid in the same manner as before. At 2 hours, monocytes floating in the culture medium or adherent to the endothelial surface had indented nuclei, a few granules, small vesicles, and abundant microvillous projections (Figure 7a). Cells of similar size and
 ultrastructure were also found in the collagen layer 8 hours after culture (Figure 7b). The vacuoles were \( \approx 0.5 \) to 1.0 \( \mu m \) in diameter and often contained a few round, electron-lucent vacuoles bounded by a unit membrane, suggesting that they were fat droplets. The size of the cells and the number of lipid droplets increased as the days elapsed. On day 4, the transmigrant cells possessed abundant cytoplasm containing a number of lipid droplets. They exhibited the characteristic morphology of macrophages, such as elongated and indented nuclei and a distorted cytoplasmic membrane with abundant microvillous projections (Figure 7c). On day 7, the size of these cells reached 50 \( \mu m \) in diameter, and they contained a large number of lipid droplets (Figure 7d). The cytoplasmic projections were not prominent in these cells. The size of the lipid droplets varied from 0.5 to 10 \( \mu m \). These lipid droplets occasionally contained a few crystalloid structures and electron-dense amorphous material. The nuclei were ovoid or irregular and usually located in the periphery of the cytoplasm. These cells were morphologically very similar to the foam cells observed in atheromatous plaques, which are regarded as monocyte-derived foam cells.\(^1,^2\)

**Discussion**

**Rabbit AECs and the Matrix Components Suitable for This Coculture System**

The in vitro system that we report here allows the quantitative analysis of monocyte transmigration, differentiation into macrophages, and foam cell formation in a matrix layer underneath a rabbit AEC monolayer. The concept of an in vitro system for the analysis of atherogenesis was originally developed by Navab, Fogelman, and colleagues\(^6^–^11\) using human AECs. Because there are several difficulties with obtaining primary human cultured AECs, we tested a primary culture of rabbit AECs. The rate of transmigration in this system is affected by the continuity of the endothelial monolayer, while the need to maintain the integrity of the tight junctions as well as other intracellular apparatus comprises the most important barrier to well-executed cell transmigration. To avoid excessive disruption of intercellular junctions, several improvements were adopted. First, we avoided excessive trypsin digestion of rabbit AECs. On each passage, a concerted effort was made to maintain the rabbit AECs in a sheetlike condition, each sheet consisting of >30
rabbit AECs. Freezing and thawing of AECs were also avoided. On examination by confocal microscopy, we often found places where the AECs had not covered the matrix layer properly when AECs of low viability had been used. We also used a combination of cultivation at low rabbit serum concentrations, which profoundly inhibited the growth of SMCs, and selection with a 96-well culture dish, which enabled us to completely eliminate SMC contamination. By means of this improved method, we established a workable AEC monolayer for the study of transmigration.

The second important problem was to define the optimal components of the matrix layer that would support the accumulation of transmigrant monocytes. Previous articles reported collagen type I or MATRIGEL as a component of the matrix layer. However, for rabbit AECs, pure collagen type I induces detachment of AECs from the well at the edge of the filter after several days of cultivation. MATRIGEL induces a change in the conformation of rabbit AECs, especially elongation. We therefore tested various combinations of available matrix proteins for the matrix layer underneath the rabbit AECs. Among these, a mixture of types I and IV collagen yielded the best monolayer, type IV collagen being an important component of the normal vascular basement membrane.

Quantitative Analysis of Monocyte/Macrophage Transmigration

To study monocyte transmigration, monocytes were separated by a combination of Ficoll-Paque gradient and a magnetic bead–associated anti-CD14 antibody. We chose to avoid methods involving adhesion molecules, which might have affected the results of the transmigration assay. Previous reports had indicated that anti-CD14 bead separation may only minimally affect monocytes. This method also enabled us to obtain \( \times 10^6 \) CD14-positive cells from 200 mL of blood from a single healthy donor. Attempts were made to develop a similar method for the separation of rabbit monocytes, but a sufficient number of pure monocytes could not be obtained by this method.

Because the number of spontaneously transmigrating monocytes was low, a treatment step with human recombinant MCP-1 was implemented. MCP-1 is the most potent chemoattractant for human monocytes, and expression of MCP-1 in human atherosclerotic lesions has been reported. Pretreatment with human MCP-1 was able to induce the transmigration of human monocytes through rabbit AECs, and many of these transmigrant human monocytes in the matrix layer survived for 7 days. Furthermore, recombinant human MCP-1 can effectively mediate the interaction between human monocytes and rabbit AECs. Compared with the survival rate of human monocytes cultivated on collagen-coated dishes, the cell survival rate improved profoundly. Extracellular matrix is known as a cell survival factor, and extracellular matrix can also modulate macrophage functions characteristic of atheroma, including the production rate of matrix metalloproteinases. Most of the monocytes that transmigrated into the matrix layer in both methods 1 and 2 differentiated into macrophage-like cells and expressed cell
surface markers reported as being characteristic of foam cells in atherosclerotic lesions, including type I and type II macrophage scavenger receptors.44

A Tool for the Study of Atherogenesis

This coculture system can support both the differentiation of transmigrant monocytes and their survival for >7 days. In a preliminary experiment, we added a significant amount of oxidized LDL to the matrix layer in method 1, and this resulted in an increase in the number of monocytes retained in the matrix layer (data not shown). The addition of oxidized LDL, as shown by the successive ultrastructural studies (Figure 7), resulted in an increase in the size of monocytes after transmigration, mainly reflecting the increase in lipid droplets in their cytoplasm. By day 4, these transmigrant cells had accumulated a significant number of lipid droplets (Figure 7c), and these droplets appeared to be surrounded by a membranous structure. On day 7, the transmigrant cells had accumulated a large number of lipid droplets (Figure 7d) and appeared similar to the foam cells found in atherosclerotic lesions in experimental animals and human subjects.

The coculture system reported in this article allows the observation and investigation of monocyte transmigration and foam cell formation, as well as quantitative analyses of these processes. However, additional improvements are still needed. At first, many of the present results were obtained using method 1, which lacks SMCs, a major cellular component in the artery wall. For the preliminary experiments, we used a coculture system with SMCs and matrix proteins secreted from SMCs (method 2). This method can also support the AEC monolayer and monocyte differentiation, but the major difference in monocytes/macrophages caused by the presence of SMCs is now under investigation. Another important problem concerned the handling of lipoproteins. Although lipid accumulation in atherosclerosis in human subjects and experimental animals has distinctive histopathological features,44,14,15,45 in this study we simply added CuSO4-modified, oxidized LDL to the matrix component.

The molecular mechanisms by which modification of lipoproteins in the arterial wall occurs still remain poorly understood. The lack of positional and signal information for vascular cells, including the role of shear stress, the oxygen gradient, the NO gradient, and stimulation by the nervous system, may prove to be important shortcomings of this system. In the presence of these stimuli, vascular cells may well behave differently. Nevertheless, whatever refinements of this system are developed in the future to bring it into closer approximation of the actual physiological conditions,
the establishment of such a system marks an important advance, in that it now makes possible the study of molecular mechanisms of foam cell formation in vitro in the presence of the main types of artery wall cells and in a special, structural setting similar to that in the artery wall. It is well known that the interaction of the 3 cell types (ie, ECs, SMCs, and monocytes/macrophages) with each other has profound effects on the metabolism and function of vessel wall components, including matrix, cytokines, and growth factors.66

In conclusion, we have developed a vascular cell coculture system that can support an AEC monolayer for up to 2 weeks, as well as foam cell formation by transmigrant human monocytes. This coculture method can provide a technology platform for future studies addressing crucial aspects of atherosclerosis.

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