Factors regulating the activities of the low density lipoprotein receptor and the scavenger receptor on human monocyte-macrophages

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Abstract Improved techniques of cell isolation resulted in 90 to 100 million monocytes from a single donor. Addition of low density lipoprotein (LDL) to cultures of these cells resulted in the down regulation of LDL receptor activity. Addition of malondialdehyde-altered LDL, which enters the cell through a receptor for negatively charged proteins (the scavenger receptor), produced an even greater down regulation of the LDL receptor, indicating that both receptors are present on the same cell. Within hours of adherence of the cells, there was a dramatic decrease in the activity of both receptors. LDL receptor activity was highest during the first week in culture and then declined, despite the maintenance of a constant LDL concentration in the medium. Scavenger receptor activity surpassed LDL receptor activity by the 6th day and was maximally expressed during the second week. Increasing cell density resulted in a slight increase in the activity of the LDL receptor and a dramatic increase in scavenger receptor activity. Insulin had no significant effect on either receptor. Removing serum from the culture medium for 48 hr resulted in a 3.5-fold increase in LDL receptor activity and a 2-fold decrease in scavenger receptor activity. Twenty-four hr after the cells were re-exposed to serum, the activities of both receptors essentially returned to base line. Heat-inactivation of serum was associated with an increased cholesteryl ester content of the cells and depressed receptor activities. Scavenger receptor activity appears related to the maturation of monocytes into macrophages and is promoted by increasing cell density and serum factors that are heat labile.—**Fogelman, A. M., M. E. Haberland, J. Seager, M. Hokom, and P. A. Edwards.** Factors regulating the activities of the low density lipoprotein receptor and the scavenger receptor on human monocyte-macrophages. *J. Lipid Res.* 1981. **22:** 1131–1141.

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We recently reported (1, 2) that human monocyte-macrophages possess both the classic low density lipoprotein (LDL) receptor and a receptor for negatively charged proteins, the scavenger receptor (3, 4). Native LDL was processed by the LDL receptor but did not produce cholesteryl ester accumulation. LDL altered by treatment with malondialdehyde (MDA-LDL) was processed by the scavenger receptor and produced cholesteryl ester accumulation similar to that seen in arterial foam cells. However, we were unable to decide from these studies whether there were two populations of cells, each with one receptor, or one population of cells with both receptors on each cell. The studies reported here indicate that there is a single population of cells with both receptors on each cell.

During the course of our experiments on these cells, we noted that the ratio of the activity of the LDL receptor to the scavenger receptor varied from approximately 1:1 to 1:10. This wide variation prompted us to make a detailed search for factors that regulate these receptor activities. In order to carry out these experiments, we often required greater numbers of cells than could be obtained by the methods that we developed earlier (5). Consequently, we devised variations of these methods that produce 90 to 100 million monocytes from a single donor. Using these cells, we found that a factor such as insulin that has been shown to affect the activity of the LDL receptor in cultured human fibroblasts (6) has no effect on either the LDL receptor or the scavenger receptor of human monocyte-macrophages. However, some factors, such as adherence to plastic, caused both receptor activities to change dramatically in the same direction while other factors, such as the presence or absence of serum, caused the two receptor activities to change in opposite directions. The results of these studies indicate

Abbreviations: LDL, low density lipoprotein; MDA-LDL, LDL modified by malondialdehyde.

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that the activities of these two receptors on human monocyte-macrophages depend on both the stage of development of the cells and the conditions of culture.

EXPERIMENTAL PROCEDURES

Materials

^{125}I as sodium iodide in dilute NaOH, pH 7–11 (sp act 15 mCi $^{125}I/\mu g$ of iodine) was purchased from Amersham, Arlington Heights, IL. Crystallized Human Albumin (Cat. No. A-8763) was purchased from Sigma Chemical Co., St. Louis, MO. Antibiotic-Antimycotic (Cat. No. 600-5245) was purchased from Grand Island Biological Co., Grand Island, NY. All other equipment and supplies were from sources previously reported (5).

Subjects

Normal subjects were recruited from the staff and student body at UCLA. No one received drugs that might have affected serum cholesterol levels or lipid metabolism. All had normal hematocrits, white blood cell and differential counts, serum cholesterol, and triglycerides. Informed consent was obtained in writing from each person.

Separation of cells

Three hundred and sixty or 500 ml of blood was taken after an overnight fast and the monocytes were separated from 180 ml of blood by Method B as previously described (2, 5) or were separated from 300 ml of blood by a variation of this method. The variations described below were developed because the previous methods resulted in an overloading of the separation chamber when more than 180 ml of blood was processed.

Method C. Sixty ml of blood was drawn into each of five syringes, heparinized, mixed with Plasmagel, allowed to settle, and the supernatant was removed as previously described (Method B in reference 5). The supernatants were centrifuged in 40-ml sterile plastic tubes at 150 *g* for 10 min at 5°C. The platelet-rich supernatant was carefully removed and discarded. The pellets from two tubes were each resuspended in 10 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 15 mM glucose and 1% bovine serum albumin (buffer A), and transferred to successive tubes until all the pellets were resuspended in two tubes, each containing 10 ml. The tubes from which the cells had been taken were washed successively with an additional 10 ml of buffer A which was added to the two tubes containing the cells. Buffer A was added to bring the total volume of the cells to 40 ml. The cells were centrifuged at 150 *g* for 10 min at 5°C and the supernatant was discarded. The cells were resuspended in 40 ml of buffer A and the 150 *g* spin was repeated. The supernatant was again discarded and the cells were resuspended in buffer A containing penicillin (100 units/ml) and streptomycin (100 μg/ml) (hereafter referred to as buffer B) and transferred to two sterile, siliconized glass, conical centrifuge tubes using 5 ml of buffer B to wash the plastic tubes. Half of the cells from each of the two siliconized glass tubes were transferred to two other siliconized glass tubes, and buffer B was added to give a volume of 25 ml in each of the four tubes. Fifteen ml of Ficoll-Paque was underlayered in each tube and the tubes were centrifuged at 400 *g* for 40 min at 18°C. The mononuclear cells at the interface of each tube were transferred to another siliconized tube containing 10 ml of buffer B at 4°C using a siliconized Pasteur pipet which was rinsed with buffer B. Buffer B was added to bring the volume to 40 ml and the tubes were centrifuged at 400 *g* for 10 min at 5°C. The supernatant was discarded and the cells were resuspended in 10 ml of buffer B by sequentially transferring the contents of each tube. The tubes were washed with an additional 10 ml of buffer B, resulting in the resuspension of all of the cells in 20 ml of buffer B. The cells were injected into the loading chamber of the Beckman Elutriator System and processed as previously described (2, 5). In 14 consecutive experiments, the monocyte fraction contained $114 \pm 39 \times 10^6$ cells of which $79 \pm 8\%$ were monocytes.

Method BB. This method differed from method B as described previously (2, 5) in that 300 ml of blood was processed for cells and the Plasmagel supernatant was centrifuged once at 150 *g* at 5°C for 10 min in order to remove the platelets. The pellets were sequentially resuspended in 20 ml of buffer A and combined into two tubes. The other tubes were washed sequentially with buffer A and the washes were added to the two tubes containing the cells. The cells were centrifuged at 400 *g* for 10 min at 5°C. The supernatant was again discarded and the pellets were resuspended in 18 ml of buffer A. One tube was kept on ice while the contents of the other was injected into the Elutriator system and processed as described previously (2, 5), except that an additional 40 ml of monocyte fraction was collected at a flow rate of 18.2 ml/min. Upon completion of the elutriation, the separation chamber and outlet tubing were removed from the system, washed first with distilled water and then with buffer A, and returned to the system. The cells that had been held on ice were then injected into the system and elutriated. The fractions from the two elutriations were combined, processed through Ficoll-

Paque, washed, and resuspended for culture as described previously (2, 5). In ten consecutive experiments, the monocyte fractions contained 110 ± 27 × 10^6 cells of which 89 ± 6% were monocytes.

Cell culture

Unless otherwise specified, the cells were suspended in 30% autologous serum in Dulbecco's modified Eagle's medium supplemented with $NaHCO_3$ (24 mM), HEPES (10 mM), insulin (8 μg/ml), glucose (2 mg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and Fungizone (0.25 μg/ml) (hereafter referred to as medium B in order to be consistent with our previous publications (1, 2)). The addition of the Fungizone had no effect on protein content or receptor activities. One-ml samples of the cell suspension containing 10^6 cells (unless otherwise specified) were transferred to 35 × 10 mm plastic tissue culture dishes and incubated at 37°C in a humidified incubator with 5% CO_2. Unless otherwise stated, the medium was aspirated and replaced with fresh medium of the same composition twice weekly.

Classification and viability of cells. The cells were classified and their viability determined as described previously (2, 5). Because of the media changes and the washes prior to beginning each experiment, the cells were ≥99% monocyte-macrophages before the radioactive lipoproteins were added, regardless of the separation method used. More than 95% of the cells were viable at the end of the incubations.

Preparation of sera and lipoproteins. Autologous serum was prepared from 180 or 200 ml of blood by placing 25-ml aliquots in 30-ml Corex tubes at room temperature. After 30 min the clots were reamed. Thirty min later the tubes were placed at 4°C. Two hr later the tubes were centrifuged at 900 *g* for 15 min at 5°C. The supernatants were removed, filtered through a 0.45-μ filter and stored at 4°C. LDL and MDA-LDL were prepared and radioiodinated as described previously (1, 2).

Assays

Prior to the addition of radioactive lipoproteins, the cells were washed three times with 1.5 ml of Dulbecco's modified Eagle's medium containing 10 mM HEPES (medium C). Radioactive lipoproteins were added in the same medium supplemented with 24 mM $NaHCO_3$ and glucose, 2 mg/ml (medium D).

The proteolytic degradation of ^{125}I-labeled native LDL and ^{125}I-labeled MDA-LDL was measured by assaying the amount of ^{125}I-labeled trichloracetic acid-soluble (noniodide) material formed by the cells and excreted into the culture medium as described by Goldstein and Brown (7). Corrections were made for the small amounts (<0.01% of total radioactivity added) of ^{125}I-labeled acid-soluble material that was found in parallel incubations without cells. The free and esterified cholesterol contents and protein contents of the cells were determined by previously described and cited methods (2). Statistical analyses were carried out by methods cited previously (5). Phase contrast photomicrographs were taken using a Leitz Diavert inverted microscope equipped with a trinocular head and a 35-mm Olympus camera attached to an Olympus photomicrograph apparatus. All photographs were taken at ×400 magnification.

RESULTS

In our previous publications (1, 2) we could not exclude the possibility that the LDL receptor was located on one subpopulation of monocytes, while the scavenger receptor was located on another. If this were the case, then addition of MDA-LDL should not lead to down regulation of the LDL receptor. However, if the two receptors were located on the same cell then the addition of MDA-LDL would be expected to lead to down regulation of the LDL receptor. Based on the work of Goldstein and Brown (3) neither the addition of native LDL or MDA-LDL would be expected to down regulate the scavenger receptor. The experiments shown in **Fig. 1,** panel B demonstrate that, as predicted, neither native LDL or MDA-LDL down-regulated the scavenger receptor. In contrast, the addition of both native LDL and MDA-LDL caused down regulation of the LDL receptor. Indeed, MDA-LDL produced significantly more down regulation than native LDL (Fig. 1, panel A), strongly suggesting that the two receptors are located on the same cell.

LDL receptor activity was previously demonstrated in freshly isolated human monocytes (see Fig. 3 in reference 2). Evidence of scavenger receptor activity has also been observed in freshly isolated monocytes (**Fig. 2,** day 0). However, this activity decreased after one day in culture (Fig. 2, Day 1). Since adherence of monocytes to a surface has been known to produce substantial changes in cellular metabolism within 2 hr (8, 9), the effects of adherence on LDL and scavenger receptor activities was studied. As shown in **Table 1,** adherence of the cell produced a profound decrease in the high-affinity degradation of both ^{125}I-labeled native-LDL and ^{125}I-labeled MDA-LDL.

In the experiments shown in Table 1, the activity of the LDL receptor was slightly higher than that of the scavenger receptor. This is very different from those experiments previously reported (see Figs. 5 and

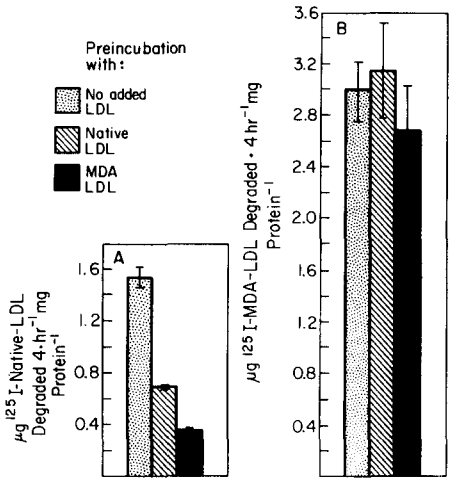

Fig. 1. The effects of preincubation with native LDL or MDA-LDL on receptor activity. Normal monocytes prepared by method C were cultured in 1 ml of 30% autologous serum in medium B. On day four, 500 μg of native LDL (cross-hatched bars) or 500 μg of MDA-LDL (solid bars) was added to some of the dishes. On day 7 the medium was removed, the cells were washed three times with 1.5 ml of medium C, and 1 ml of medium D containing either 30 μg ^{125}I-labeled native LDL (232 cpm/ng protein) with or without a 50-fold excess of nonradioactive native LDL (panel A) or 10 μg ^{125}I-labeled MDA-LDL (232 cpm/ng protein) with or without a 50-fold excess of nonradioactive MDA-LDL (panel B) was added. After 4 hr of incubation at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The values obtained in the presence of a 50-fold excess of nonradioactive lipoprotein were substracted from the values obtained in the absence of nonradioactive lipoproteins. The values shown are the mean ±1 SD of quadruplicate dishes.

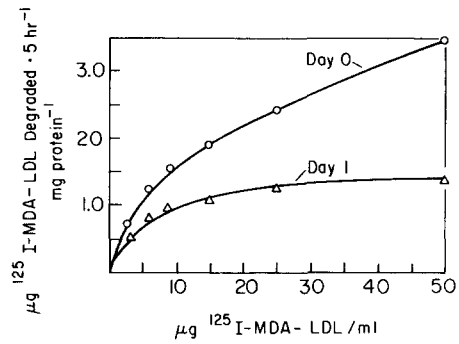

Fig. 2. ^{125}I-labeled MDA-LDL degradation by human monocytes and macrophages. Normal monocytes were prepared by method B and some were incubated on a surface to which they could not adhere (Teflon FEP) in medium D containing the concentrations of ^{125}I-labeled MDA-LDL protein (87 cpm/ng protein) shown on the abscissa. After 5 hr at 37°C, the incubation mixture was centrifuged at 1,750 *g* for 10 min at 5°C and the content of ^{125}I-labeled acid-soluble material in the medium was determined (day 0, circles). The remainder of the monocytes were cultured in plastic petri dishes in 30% autologous serum in medium B. The next day the medium was removed, the cells were washed three times with 1.5 ml of medium C, and 1 ml of medium D and the concentration of ^{125}I-labeled MDA-LDL protein (87 cpm/ng protein) shown on the abscissa was added. After 5 hr at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined (day 1, triangles).

6 in reference 2) where scavenger receptor activity was 7- to 10-fold greater than LDL receptor activity. Since ^{125}I-labeled MDA-LDL appeared to be degraded by a high-affinity saturable process with maximal velocity at 10–15 μg of protein/ml (see Fig. 4 in reference 1 and Fig. 2 in this paper) while ^{125}I-labeled native LDL appeared to be degraded by a saturable high-affinity process with maximal velocity at 25–50 μg protein/ml (1, 2), it appeared reasonable to compare the relative activity of these two receptors at the lipoprotein concentrations used in the experiments described in Table 1. When the data from many experiments using different subjects and cells prepared by different methods were plotted as a function of time (**Fig. 3**), the following patterns emerged. During the

TABLE 1. The effect of adherence on the degradation of ^{125}I-labeled native LDL and ^{125}I-labeled MDA-LDL

Condition	^{125}I-labeled Native LDL Degradation			^{125}I-labeled MDA-LDL Degradation		
	Total (a)	Non-specific (b)	High-affinity (a–b)	Total (c)	Non-specific (d)	High-affinity (c–d)
	μg degraded·5 hr^{-1} mg protein^{-1}					
Non adhered	2.16 ± 0.30	0.17	1.99	1.64 ± 0.15	0.16	1.48
Adhered	0.63 ± 0.02	0.04	0.59	0.40 ± 0.06	0.03	0.37

Normal monocytes were prepared by method B and resuspended in 30% autologous serum in medium B. Half of the cells were transferred to plastic petri dishes and incubated at 37°C. The other cells were washed three times in medium C with centrifugation at 5°C and kept on ice. After 2 hr the cells in the plastic petri dishes were washed three times with 1.5 ml of ice-cold medium C, and 1 ml of medium D containing either 20 μg ^{125}I-labeled native LDL (79 cpm/ng protein) with or without a 50-fold excess of nonradioactive LDL or 10 μg of ^{125}I-labeled MDA-LDL (79 cpm/ng protein) with or without a 50-fold excess of nonradioactive MDA-LDL was added. The washed monocytes, which had been kept on ice, were incubated on a surface to which they could not adhere (Teflon FEP) in medium D containing the same concentrations of lipoproteins that were in the plastic petri dishes. After 5 hr at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined as described in the legend to Fig. 2. Total degradation and nonspecific degradation represent the values obtained in the absence or presence, respectively, of a 50-fold excess of nonradioactive lipoprotein. The values shown for total degradation are the mean ± 1 SD of quadruplicate dishes and the values shown for nonspecific degradation are the average of duplicate dishes.

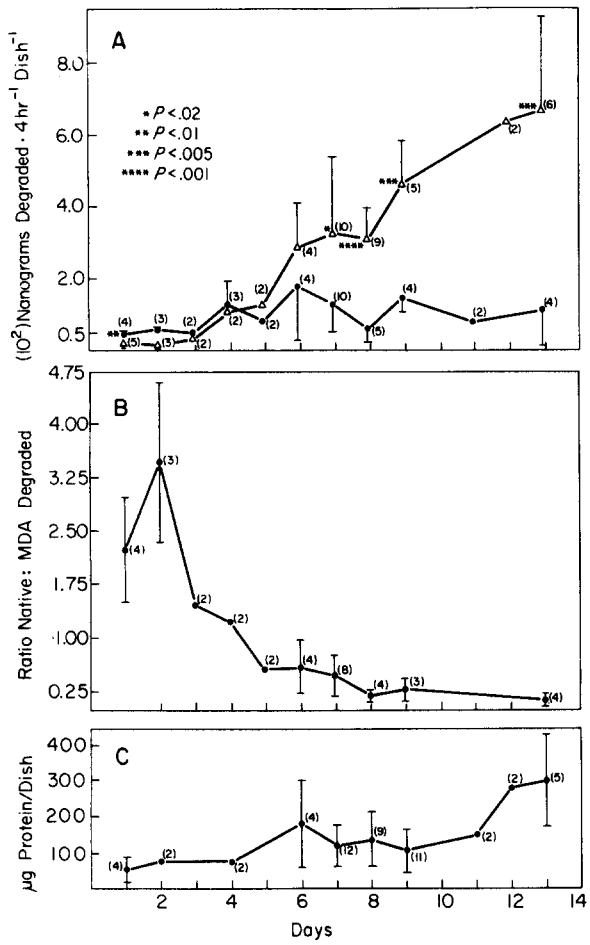

Fig. 3. LDL and scavenger receptor activities as a function of time in culture. Data from experiments using different normal subjects and cells prepared by methods B, C, or BB are plotted as a function of time in culture. In each instance, 1×10^6 monocytes were cultured in 1 ml of 30% autologous serum in medium B in plastic petri dishes. After the period of time shown on the abscissa, the medium was removed, the cells were washed three times with 1.5 ml of medium C, and 1 ml of medium D containing either 20 µg ^{125}I-labeled native LDL (circles) or 10 µg ^{125}I-labeled MDA-LDL (triangles) was added. In some experiments the radioactive lipoproteins were added in the presence or absence of a 50-fold excess of nonradioactive lipoprotein. After 4 hr at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The values shown are the mean ± 1 SD. Each experiment was done with duplicate or quadruplicate dishes. The values in parentheses represent the number of experiments. The data in panel A represent total degradation per dish (i.e., degradation in the absence of excess nonradioactive lipoprotein). From experiments in which degradation was measured in the presence or absence of a 50-fold excess of nonradioactive lipoprotein, it was determined that 94 ± 2% and 92 ± 3% of the total degradation of ^{125}I-labeled native LDL and ^{125}I-labeled MDA-LDL, respectively, was due to high-affinity degradation. Panel B represents the ratio of degradation of ^{125}I-labeled native LDL to ^{125}I-labeled MDA-LDL in experiments in which the two lipoproteins were degraded by the same cells. Panel C depicts the protein content of the dishes.

first 3 days of culture the activity of both receptors was low but LDL receptor activity exceeded scavenger receptor activity (Fig. 3, panels A and B). In general, LDL receptor activity was greatest at about the sixth day and then declined despite the maintenance of a constant LDL concentration in the medium (Fig. 3, panel A). Scavenger receptor activity surpassed LDL receptor activity by the sixth day and was maximally expressed during the second week (Fig. 3, panel A). The ratio of the two activities calculated from experiments made on the same cells is shown in Fig. 3, panel B. The protein concentration of the dishes is shown in panel C. An example of the time course for both receptors on cells from the same subject is shown in **Fig. 4.** In this experiment, LDL receptor activity did not increase during the first week in culture as often was the case (see Fig. 3 in reference 2 and Fig. 3, panel A in this report). However, in this example as in virtually all others, LDL receptor activity declined after the first week despite the maintenance of a constant LDL concentration in the medium. **Fig. 5** demonstrates the marked morphological changes that the cells undergo in culture. After 2 hr, the cells were spread on the dish more than at 24 hr indicating retraction after the initial adherence. Between the third and sixth days, the cells increased in size and developed large cytoplasmic veils, and continued to increase in size during the second week.

Plasmagel, a commercially available gelatin solution (6 mg gelatin/ml) was used in preparing the cells de-

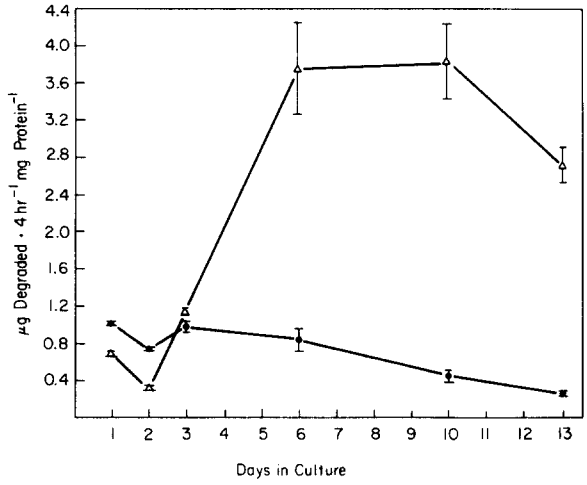

Fig. 4. LDL and scavenger receptor activities in cells from a single subject as a function of time in culture. Normal monocytes were prepared by method BB and were cultured in 1 ml of 30% autologous serum in medium B. After the period of time shown on the abscissa, the medium was removed, the cells were washed three times with 1.5 ml of medium C, and 1 ml of medium D containing either 20 µg ^{125}I-labeled native LDL (200 cpm/ng protein) (circles) or 10 µg ^{125}I-labeled MDA-LDL (189 cpm/ng protein) (triangles) was added. After 4 hr at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The values shown are the mean ± 1 SD of quadruplicate dishes.

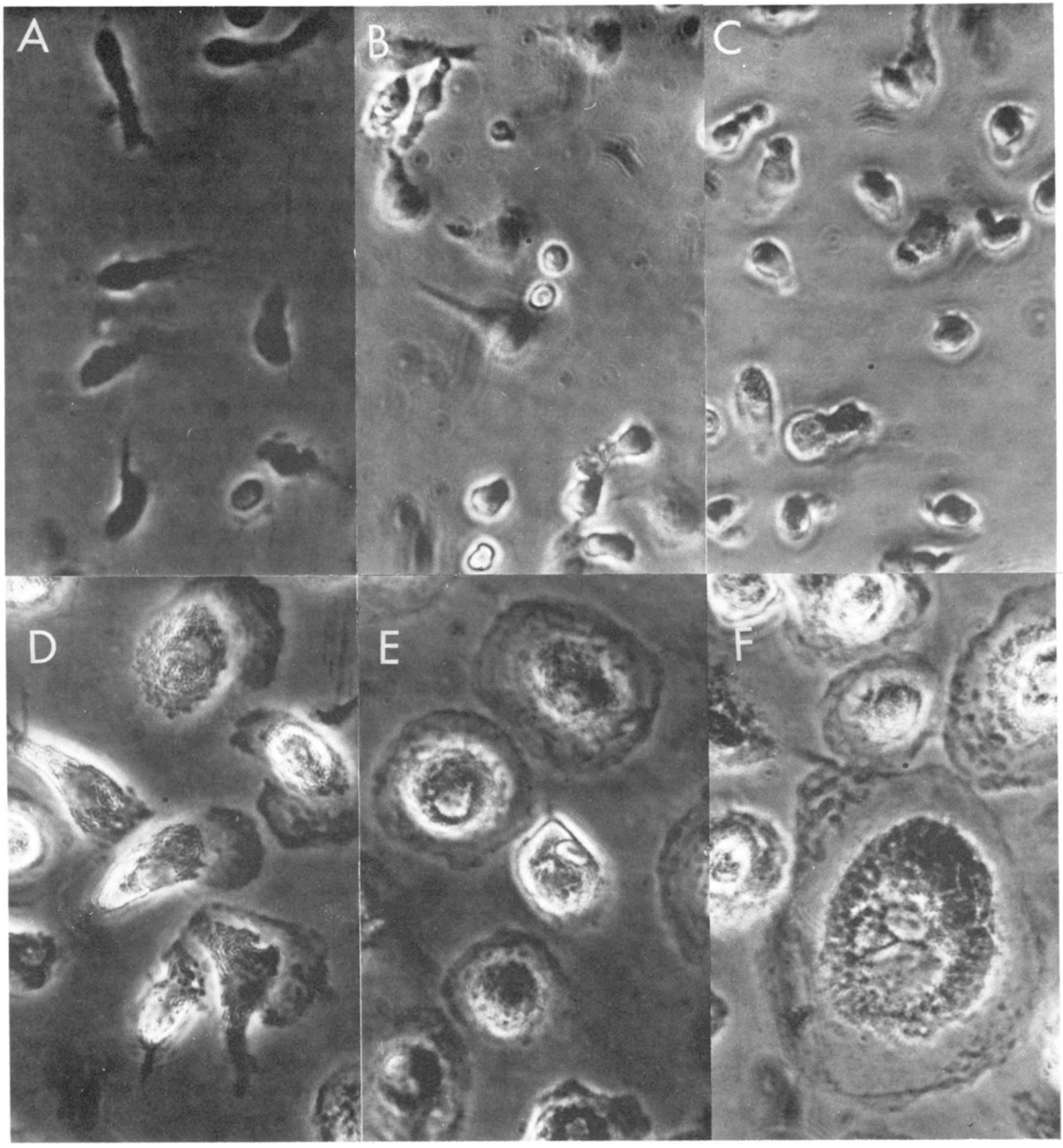

Fig. 5. The morphology of human monocyte-macrophages as a function of time in culture. The cells described in Fig. 4 were photographed in situ through a phase contrast microscope at ×400 magnification (reduced for reproduction) after 2 hr in culture (A), 1 day in culture (B), 3 days in culture (C), 6 days in culture (D), 10 days in culture (E), and 13 days in culture (F).

scribed in this report and in previous reports (1, 2, 5, 10–14). This product is made by a French company (Roger Bellon Laboratories, Neuilly, France) and is distributed by HTI Corp., Buffalo, NY. The manufacturer has consistently refused to divulge the source of the gelatin used in the formulation. In our earlier reports (10–12), Plasmagel was indeed a gel at 4°C. However, in later years (1, 2, 5, 13, 14) it was a

liquid at 4°C.[3] These characteristics fit very well the properties of gelatin solutions made from different species of sea weed that produce carrageenan (15), a substance known to compete effectively for the binding site (3) on the scavenger receptor at concentrations of 20 μg/ml (4). It, therefore, became essential to determine if the low scavenger receptor activities observed during the first few days in culture (Figs. 4 and 5) could have been due to the presence of an inhibitor in the Plasmagel that was carried forward from the isolation procedure. This was not the case, as there was no difference in receptor activities whether the cells were isolated with or without Plasmagel (data not shown).

Stein and Stein (16), Kurth et al. (17), and Fielding et al. (18) demonstrated that cell density was an important determinant of LDL receptor activity in cultured rat aortic smooth muscle cells, human fibroblasts, and bovine endothelial cells, respectively. The effect of cell density on LDL and scavenger receptor activities in human monocyte-macrophages is shown in **Fig. 6.** In this experiment there was little effect on the LDL receptor (Fig. 6, panel B) while there was a substantial effect on the scavenger receptor (Fig. 6, panel C). In other experiments not shown here, increasing cell density produced a slightly greater increase in LDL receptor activity than was the case in this experiment (Fig. 6, panel B). However, in every instance (four out of four experiments) increasing cell density produced a much greater increase in scavenger receptor activity.[3]

Chait, Bierman, and Albers (6) demonstrated that insulin increased LDL receptor activity in cultured human fibroblasts. Since insulin has been routinely included in our culture media (1, 2), we were previously unable to exclude the possibility that the relatively high LDL receptor activities observed in our cells was due to the inclusion of insulin in the medium (2). Experiments made with or without insulin demonstrated that this was not the case (data not shown). Moreover, insulin did not have a significant effect on scavenger receptor activity or on the protein content of the dishes (data not shown).

The experiments in **Fig. 7** demonstrate that serum has a highly significant effect on both the LDL and scavenger receptor activities. After 5 days in culture, some of the cells were switched to a medium containing no serum. Forty-eight hr later the activity of the scavenger receptor was substantially higher than that of the LDL receptor in the cells that had been

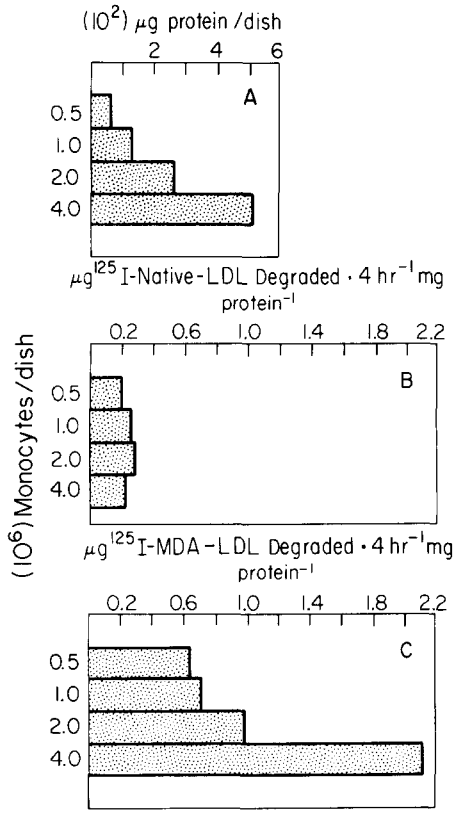

Fig. 6. The effect of cell density on LDL and scavenger receptor activities. Normal monocytes were prepared by method C and cultured in 1 ml of 30% autologous serum in medium B in plastic petri dishes at the cell density shown on the ordinate. After 7 days the medium was removed, the cells were wahsed three times with 1.5 ml of medium C, and 1 ml of medium D containing either 20 μg ^{125}I-labeled native LDL (189 cpm/ng protein) with or without a 50-fold excess of nonradioactive native LDL (panel B) or 10 μg ^{125}I-labeled MDA-LDL (187 cpm/ng protein) with or without a 50-fold excess of nonradioactive MDA-LDL (panel C) was added. After 4 hr of incubation at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The values obtained in the presence of a 50-fold excess of nonradioactive lipoprotein were subtracted from the values obtained in the absence of nonradioactive lipoprotein. The values shown are the average of duplicate dishes. The protein content of the dishes is shown in panel A.

continuously exposed to 30% autologous serum (Fig. 7, panel A, 30% AS). However, the opposite was true for the cells that had been maintained for 2 days in a medium without serum (Fig. 7, panel A, 0.1% HSA). In the absence of serum there was an approximately 3.5-fold increase in the LDL receptor activity and an approximately 2-fold decrease in the scavenger receptor activity. The protein content of the cells that had been maintained in 30% autologous serum (Fig. 7, panel A, 30% AS) was 130 ± 24 μg/dish, while the protein content of the cells that had been maintained for 48 hr in the medium without serum (Fig. 7, panel A, 0.1% HSA) was 68 ± 5 μg/dish. Some of

[3] Fogelman, A. M., M. E. Haberland, J. Seager, M. Hokom, and P. A. Edwards. Unpublished observations.

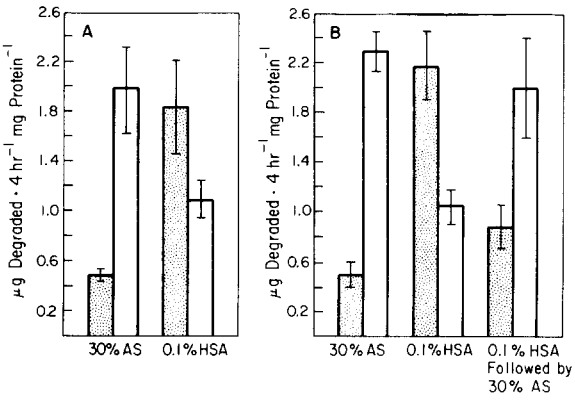

Fig. 7. The effect of serum on LDL and scavenger receptor activities. Normal monocytes were prepared by method BB and cultured in 1 ml of 30% autologous serum in medium B. After 5 days the medium was removed, the cells were washed three times with 1.5 ml medium B (without bicarbonate), and 1 ml of 30% autologous serum in medium B (30% AS) or 1 ml of medium B with 0.1% human serum albumin (0.1% HSA) was added. Forty-eight hr later, the medium was removed from some of the dishes, the cells were washed three times with 1.5 ml medium C, and 1 ml of medium D with either 20 μg ^{125}I-labeled native LDL (415 cpm/ng protein) (panel A, stippled bars) or 10 μg ^{125}I-labeled MDA-LDL (432 cpm/ng protein) (panel A, open bars) was added. After 4 hr of incubation at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The medium was removed from the remaining cells that had been continuously maintained in 30% autologous serum in medium B and replaced with 1 ml of the same medium (panel B, 30% AS). The medium was also removed from the remaining cells that had been maintained for 48 hr in medium B with 0.1% human serum albumin and half of the cells received 1 ml of the same medium (panel B, 0.1% HSA), while the other half received 1 ml of 30% autologous serum in medium B (panel B, 0.1% HSA followed by 30% AS). Twenty-four hr later the medium was removed, the cells were washed three times with 1.5 ml of medium C, and 1 ml of medium D with either 20 μg ^{125}I-labeled native LDL (415 cpm/ng protein) (panel B, stippled bars) or 10 μg ^{125}I-labeled MDA-LDL (432 cpm/ng protein) (panel B, open bars) was added. After 4 hr of incubation at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The values shown are the mean ± 1 SD of quadruplicate dishes.

the cells that had been maintained for 48 hr in the medium without serum were continued in the same medium for another 24 hr (Fig. 7, panel B, 0.1% HSA), while some of these cells were switched back to a medium containing 30% autologous serum (Fig. 7, panel B, 0.1% HSA followed by 30% AS) and compared to cells that had been continuously incubated in 30% autologous serum (Fig. 7, panel B, 30% AS). Within 24 hr of being re-exposed to serum, the LDL receptor activity decreased by approximately 2.5-fold while the scavenger receptor activity increased by approximately 2-fold (Fig. 7, panel B). The protein contents of the dishes that had been continuously exposed to autologous serum (Fig. 7, panel B, 30% AS) or maintained for 72 hr in a medium without serum (Fig. 7, panel B, 0.1% HSA) or maintained in a medium without serum for 48 hr and then re-exposed to medium containing 30% autologous serum for 24 hr (Fig. 7, panel B, 0.1% HSA followed by 30% AS) were 160 ± 14 μg/dish, 57 ± 4 μg/dish, and 89 ± 8 μg/dish, respectively.

The cells that had been exposed for 48 hr to the medium without serum changed morphologically, in most instances losing their cytoplasmic veils and taking on the appearance of tree trunks or branches but in some instances appearing to swell. The reversal of these changes after 24 hr re-exposure to serum was virtually complete.

In an attempt to characterize the factor(s) in serum that alter receptor activity, the effect of heat-inactivation was studied. As shown in **Fig. 8,** cells maintained 7 days in serum that was heated to 56°C for 30 min had depressed receptor activities, the LDL receptor being affected more profoundly. The protein content of the dishes incubated with the heated serum was 286 ± 21 μg protein/dish while the protein content of the dishes incubated with serum that was not heated was 223 ± 11 μg protein/dish. As shown in **Fig. 9,** heat-inactivation of serum produced an almost 5-fold increase in the cholesteryl ester content of the cells. This probably resulted from inactivation of lecithin:cholesterol acyltransferase activity in the serum (19, 20). The reason for the decrease in scavenger receptor activity is not apparent.

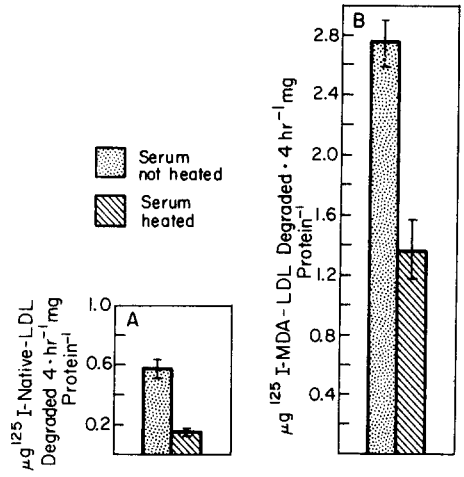

Fig. 8. The effect of heat-inactivation of serum on LDL and scavenger receptor activities. Normal monocytes were prepared by method BB and cultured in 1 ml of 30% autologous serum in medium B. In half the dishes the autologous serum had been heated to 56°C for 30 min (serum heated, cross hatched bars) and in the other half it had not been heated (serum not heated, stippled bars). After 7 days the medium was removed, the cells washed three times with 1.5 ml medium C, and 1 ml of medium D containing either 20 μg ^{125}I-labeled native LDL (194 cpm/ng protein) (panel A) or 10 μg ^{125}I-labeled MDA-LDL (180 cpm/ng protein) (panel B) was added. After 4 hr at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The values shown are the mean ± 1 SD of quadruplicate dishes.

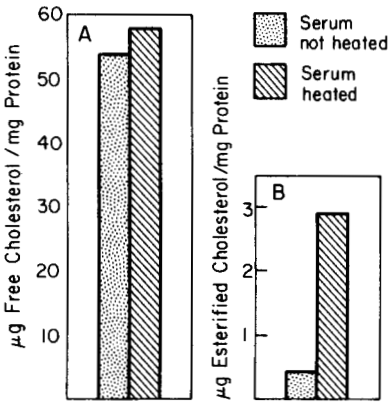

Fig. 9. The effect of heat inactivation of serum on the free and esterified cholesterol content of human monocyte-macrophages. Normal monocytes were prepared by method B and cultured in 1 ml of 50% autologous serum (serum not heated, stippled bars) or in 1 ml of 50% autologous serum that had been heated to 58°C for 30 min (serum heated, cross hatched bars). After 15 days the medium was removed, the cells were washed in the dishes, harvested, washed, and their free (panel A) and esterified (panel B) cholesterol contents were determined. The values shown are the average of duplicate determinations on the cells from four dishes.

DISCUSSION

The variations reported here of our previously published (5) methods for cell isolation produced 90–100 million monocytes from 300 ml of blood. This represents a 2- to 4-fold increase in the number of monocytes obtained from a single donor (5). Approximately 2 weeks after plating a million monocytes per 35 × 10 mm plastic petri dish there was an average of 250 μg protein/dish or an average of 25 mg per donor with method BB. This compares favorably with the yield recently reported by Traber and Kayden (21) who cultured 3–5 × 10^9 mononuclear cells that were obtained as a byproduct of plateletphoresis of multiple donors. One can calculate that culture of these cells produced no more than 26 mg of protein. Thus the material obtained is approximately the same for the two methods. Traber and Kayden's method (21) has the advantage of using cells that are a byproduct of another process but has the disadvantage of providing a mixture of cells, taken from different donors, which must be maintained in pooled sera made from yet other donors. The methods reported here permit the study of the cells from a single subject maintained in that subject's own serum.

The evidence presented in this report indicates that the LDL receptor and the scavenger receptor are on the same monocyte. The activity of both receptors decreases within hours of adherence of the cell to a surface, but the activity of the LDL receptor remains approximately equal to, or is even greater than, that of the scavenger receptor. During the first week of culture, LDL receptor activity either remains constant or increases, but invariably declines during the second week of culture. However, the scavenger receptor is fully expressed only during the second week, suggesting that this receptor may serve a function on the macrophage that is not required on the monocyte. The development of scavenger receptor activity as a function of time in culture is strikingly similar to the development of receptor activity for the third component of complement (C3) during in vitro maturation of human monocytes into macrophages (see Fig. 4 in reference 22). The relationship (if any) between C3 receptor function and scavenger receptor activity is currently under investigation.

In contrast to cultured rat aortic smooth muscle cells, human fibroblasts, and bovine endothelial cells (16–18), LDL receptor activity on human monocyte-macrophages does not decline with increasing cell density and the activity of the scavenger receptor clearly increases with increasing cell density. The failure of insulin to affect LDL receptor activity in human monocyte-macrophages points out the difficulty in generalizing from one cell type to another. The experiments described in Fig. 1 and those reported by Traber and Kayden (21) would suggest that LDL receptor activity in these cells is related to the concentration of LDL in the medium. However, the LDL concentration was constant in the experiments described in Figs. 3 and 4 and yet receptor activity varied considerably. The experiments described in Figs. 8 and 9 would suggest that the cholesterol content (particularly the esterified cholesterol content) of the cells may regulate LDL receptor activity. However, the changes in receptor activity with adherence and the changes in receptor activity as a function of time in culture are not correlated with measurable changes in the free or esterified cholesterol content of the cells. We previously demonstrated that the free cholesterol content of the cells progressively increased as a function of time in culture, while the esterified cholesterol content changed very little (2). Therefore, LDL receptor activity can vary independently of these parameters. This may be most consistent with the activity of the LDL receptor being controlled by the levels of free cholesterol in a small regulatory pool (23).

Scavenger receptor activity is not affected by the concentration of normal or altered lipoproteins in the medium (see Fig. 1 and reference 3). However, scavenger receptor activity is affected by other serum components which are stimulatory (Fig. 7) and heat-labile (Fig. 8). If this receptor is important in the

genesis of atherosclerosis, then regulation of its activity by serum components could have clinical significance. However, in contrast to the case for β-VLDL (24, 25), as of this date there is no evidence that lipoproteins that are generated in vivo enter macrophages via the scavenger receptor. Proof of the existence of such molecules will be difficult to obtain because they are cleared from the circulation so rapidly (26). Additionally, the physiological function of the scavenger receptor has yet to be established. Nonetheless, the hypothesis that such molecules are generated at the site of the atherosclerotic reaction (1) remains attractive. The scavenger receptor has been demonstrated to be present on the monocytes of receptor negative homozygous familial hypercholesterolemics (4), thereby providing a potential mechanism for the cholesteryl ester accumulation that results in vivo. Moreover, platelets from persons with type IIA hypercholesterolemia (27) and from persons selected solely because of angina pectoris (28) produce more malondialdehyde than platelets from normal individuals. Malondialdehyde and glucose are two substances produced in vivo that can alter LDL in vitro so that it is processed by the scavenger receptor (2, 29).

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