

Low Density Lipoproteins Transfer Bacterial Lipopolysaccharides across Endothelial Monolayers in a Biologically Active Form

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Abstract

Rabbit aortic endothelial cells (RAECs) were grown on micropore filters in a device that allowed in situ determination of transendothelial electrical resistance (TEER). Incubation of confluent RAEC monolayers with $2 \text{ ng} \cdot \text{ml}^{-1}$ of bacterial LPS for 3 h did not change the protein content or the number of cells on the filters, but resulted in a marked decline in TEER (from 14.1 ± 0.9 to $5.1 \pm 0.6 \Omega \cdot \text{cm}^2$) and a significant increase in LDL transport across the monolayers (from 154 ± 13 to $456 \pm 41 \text{ ng} \cdot \text{h}^{-1} \text{ per cm}^2$). In contrast, exposure of RAEC monolayers for 3 d to as much as $5 \mu\text{g} \cdot \text{ml}^{-1}$ of LPS complexed to LDL (LPS-LDL) did not alter the TEER or LDL transport. LPS-LDL was transported across the monolayers at the same rate as LDL. While microgram quantities of LPS complexed to LDL did not disrupt the integrity of the endothelial monolayer, incubation of RAECs with transported LPS-LDL at concentrations of 25–100 $\text{ng LPS} \cdot \text{ml}^{-1}$ resulted in a two- to ninefold increase in the secretion of monocyte chemotactic activity by these cells. Incubation of rabbit aortic smooth muscle cells with transported LPS-LDL at concentrations of 25–100 $\text{ng LPS} \cdot \text{ml}^{-1}$ resulted in a two- to threefold increase in the secretion of monocyte chemotactic activity. We propose that LDL protects endothelial cells from the acute toxicity of LPS but the resulting complexes are transported across the endothelium in a biologically active form that can initiate an inflammatory response.

Introduction

Bacterial LPS have been shown to have pronounced cytotoxic effects on aortic endothelial cells in several species (1–3). Preliminary studies from this laboratory (4) and the studies of Morel et al. (5) indicated that the presence of lipoproteins in the incubation medium of rabbit or bovine aortic endothelial cells markedly reduced the cytotoxic effects of LPS. Ulevitch and Johnston originally reported that incubation of LPS with serum resulted in a shift in the hydrated density of LPS and modification of its endotoxic properties (6). Subsequently, in

studies conducted of species in which HDL carry most of the plasma cholesterol, it was concluded that LPS interacted with lipoproteins, particularly HDL (7). We recently demonstrated that derivatized radioiodinated LPS bound to all classes of lipoproteins, and in the presence of hyperlipidemia most of the LPS distributed to the cholesterol-rich apolipoprotein B-containing lipoproteins (8). To gain more insight into the interaction of lipoproteins, bacterial LPS, and endothelial cells, we utilized a new system (9) to study the effects of *Escherichia coli* strain 0111:B4 LPS on confluent monolayers of rabbit aortic endothelial cells (RAECs)¹ grown on micropore filters. We demonstrate that (a) LPS in the absence of lipoproteins can disrupt the integrity of RAEC monolayers at a concentration of $2 \text{ ng} \cdot \text{ml}^{-1}$; (b) LPS at even 1,000-fold higher concentrations when complexed to LDL does not disrupt the integrity of RAEC monolayers; (c) LPS-LDL complexes are transported across the monolayer at a rate similar to that of the lipoprotein alone; and (d) the transported complex is biologically active and capable of initiating an inflammatory response.

Methods

Materials. DME was purchased from M. A. Bioproducts, Walkersville, MD (catalogue 127088). Defined FCS was obtained from HyClone Laboratories, Logan, UT (catalogue A-111L). Bacterial LPS derived from *E. coli* strain 0111:B4 was purchased from List Biological Laboratories, Campbell, CA (catalogue 201). Endogenously ³H-labeled LPS, specific activity of $5,500 \text{ cpm} \cdot \mu\text{g}^{-1}$ from *Salmonella minnesota* strain Re595 was kindly provided by Dr. Peter Tobias, Scripps Clinic and Research Institute, La Jolla, CA. All other reagents and supplies were from sources previously reported (8, 9).

Endothelial monolayers. Aortic endothelial cells were isolated from New Zealand White (NZW) rabbits. The cells were grown in DME containing 15% FCS as previously reported (9). In brief, the cells were grown on micropore filters impregnated with gelatin and coated with fibronectin. Based on results from assays of Factor VIII-related antigen, silver staining, transmission electron microscopy, and freeze-fracture studies, endothelial monolayers prepared in this manner had striking similarities to the rabbit aortic endothelium in situ (9). Monolayers on filters were housed in a special device that forms U-shaped tubes with a separate compartment on each side of the filter (9). These wells accommodated growth medium or medium containing the material under study.

1. **Abbreviations used in this paper:** CM, conditioned medium; HPF, high power field; LPDS, lipoprotein-deficient serum; MDA-LDL, malondialdehyde-LDL; NZW, New Zealand White; RAECs, rabbit aortic endothelial cells; RASMCs, rabbit aortic smooth muscle cells; TEER, transendothelial electrical resistance; WHHL, Watanabe heritable hyperlipidemic.

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Monocytes. Human monocytes were obtained by counterflow centrifugation, used freshly or were cultured, and their viability was determined as previously reported (10).

Smooth muscle cells. Rabbit aortic smooth muscle cells (RASMCs) were obtained by the explant method (11) or by enzymatic digestion (12) and passaged in DME containing 10% FCS. Cells from the third or fourth passage were used for obtaining conditioned medium (CM).

Lipoproteins. LDL ($d = 1.019\text{--}1.063 \text{ g} \cdot \text{ml}^{-1}$) was isolated by the method of Havel et al. (13) from either human or rabbit plasma after a 15-h fast. LDL was radiolabeled as described (14, 15). Malondialdehyde-LDL (MDA-LDL) was prepared as described (16), and radiolabeled as for LDL.

LPS-LDL complexes. To prepare ^{125}I -LPS-lipoprotein complexes, the *E. coli* LPS was first derivatized with *p*-OH methylbenzimidate (17). The modified LPS was then radioiodinated as described by Ulevitch (17). To prepare the LPS-LDL complexes, 5 mg of ^{125}I -LPS or 0.1 mg of [^3H]LPS was incubated with 5 or 0.1 mg of LDL-protein, respectively, in 10 ml lipoprotein-deficient serum (LPDS, $d > 1.21 \text{ g} \cdot \text{ml}^{-1}$) and the LPS-LDL complexes isolated as described (8).

Electrical resistance. Transendothelial electrical resistance (TEER) was measured in situ using the device previously described (9). TEER values were determined before and after each experiment.

LDL and LPS-LDL transport. The amount of LDL or LPS-LDL transported across the RAEC monolayers was determined as described for LDL (9).

Cytotoxicity. Confluent cell monolayers were washed with DME containing 4.5 mg/ml of LPDS-protein (LPDS-DME). Tritiated or radioiodinated LPS or LPS-LDL were added to the apical side of the monolayers (cell side of the filter) containing LPDS-DME. The opposite side received LPDS-DME alone. The device containing the monolayers was placed in a 37°C incubator. At intervals TEER was measured, the media on both sides of the filter were removed, and the monolayers were washed with LPDS-DME at 37°C. LDL transport across the monolayers was then determined.

Effect of LPS-LDL on macrophages. Radiolabeled LPS-LDL (25 μg LPS $\cdot \text{ml}^{-1}$ medium) was placed on the apical side of the confluent RAEC monolayers. Transport was allowed to proceed on a shaker in a 37°C incubator for 1 h. The medium on the basal side containing the transported material was removed, filtered, and LPS-LDL at 50 ng LPS $\cdot \text{ml}^{-1}$ was incubated with 2-d-old monocyte macrophages. After 72 h, scavenger receptor activity was determined by measuring acid-soluble radioactivity produced by proteolytic degradation of ^{125}I -MDA-LDL (10, 18).

Monocyte chemotaxis. RAECs and RASMCs were grown to confluence in 24-well tissue culture plates and were incubated for 48 h with LPS-LDL that had previously been transported across confluent RAEC monolayers. The incubation medium contained LPS-LDL at concentrations of 0–100 ng LPS $\cdot \text{ml}^{-1}$ of growth medium containing 10 or 15% heat-inactivated FCS. The incubation was continued for the next 24 h with the same concentrations of LPS-LDL but with only 0.5% FCS in the medium. At the end of this incubation period, the media were removed, cell debris were separated by centrifugation, and the supernatants (the CM) were collected and tested for monocyte chemotactic activity. Unconditioned media were treated in exactly the same manner except for being incubated in the absence of cell monolayers. Chemotaxis assays were performed using Neuro Probe chambers (Neuro Probe Inc., Cabin John, MD) basically as described (19).

Other assays. The protein content of cells and lipoproteins were determined by the method of Lowry et al. (20). Cell numbers were determined electronically (Coulter Electronics Inc., Hialeah, FL).

Results

The RAEC monolayers showed a maximum electrical resistance 7 d after seeding on filters. The presence of confluent monolayers reduced the transport of LDL by > 50-fold (from $9,295 \pm 785$ to 170 ± 16 ng LDL transported $\cdot \text{h}^{-1}$ per cm^2).

Exposure of RAEC monolayers to nanogram levels of *E. coli* 0111:B4 LPS in medium containing LPDS for 3 h resulted in a dose-dependent reduction in TEER and an increase in LDL transport (Fig. 1 A and B) without a significant change in the protein content or the number of cells on the filters (53.1 ± 5.6 vs. $49.7 \pm 5.7 \mu\text{g}$ protein per cm^2 , and 109 ± 11 vs. $105 \pm 13 \times 10^3$ cells per cm^2). However, when LPS complexed to LDL at concentrations as high as $5 \mu\text{g}$ LPS $\cdot \text{ml}^{-1}$ of medium was incubated with the cells no significant changes in the electrical resistance or LDL transport were observed (Fig. 2, A and B). In other experiments addition of LPS-LDL at LPS concentrations of 25 μg LPS $\cdot \text{ml}^{-1}$ for short incubations (2 h) or 5 μg LPS $\cdot \text{ml}^{-1}$ for long incubations (3 d) had no effect on TEER and lipoprotein transport across the endothelial monolayers (data not shown). There was no significant change in the protein content or the number of cells on the filters after treatment with LPS-LDL (data not shown).

The above experiments were conducted with derivatized, iodinated LPS (^{125}I -LPS-LDL) as was the case in our previous publication (8). To exclude the possibility that our results were related to the derivatization or iodination, we determined the distribution of endogenously labeled ^3H -LPS among the lipoprotein classes and studied the effect of [^3H]LPS and [^3H]LPS-LDL on TEER and LDL transport. As shown in Table I the results obtained with the endogenously labeled LPS confirm those previously reported (8) for the distribution of LPS among the lipoprotein classes. Moreover, endogenously labeled [^3H]LPS and [^3H]LPS-LDL had similar effects to those shown in Fig. 2, A and B (data not shown).

^{125}I -LPS-LDL was transported across the RAEC monolayers at a rate similar to that for LDL (225 ± 56 and 253 ± 42 ng protein $\cdot \text{h}^{-1}$ per cm^2 for LDL or LPS-LDL, respectively). Moreover, there was no significant difference in results obtained whether human or rabbit LDL was used (data not shown).

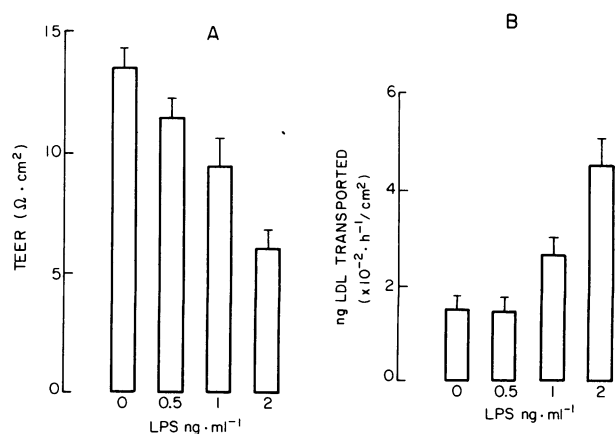


Figure 1. Effect of LPS on electrical resistance and LDL transport across endothelial monolayers. Confluent RAEC monolayers were washed with LPDS-DME and were incubated with 0.5, 1.0, and 2.0 ng/ml of *E. coli* 0111:B4 LPS in LPDS-DME. After 3 h the electrical resistance was measured (A). The monolayers were then washed and ^{125}I -LDL (100 μg LDL protein $\cdot \text{ml}^{-1}$, 60,000 cpm $\cdot \mu\text{g}^{-1}$) was placed on the endothelial side of the filters and transport allowed to proceed at 37°C. After 60 min, the amount of ^{125}I -LDL that crossed the monolayer was measured (B). The values shown are the mean \pm 1 SD of triplicate determinations.

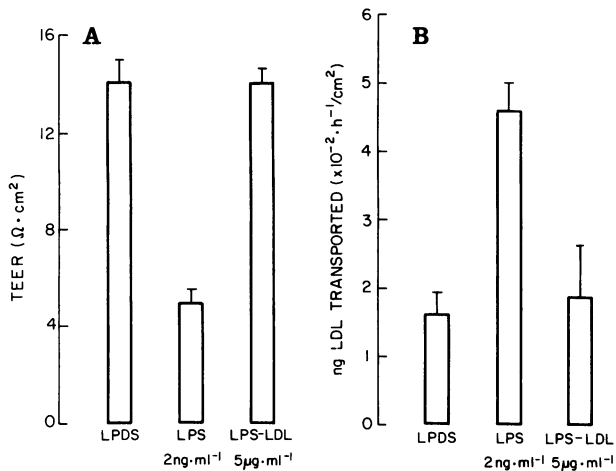


Figure 2. Effects of LPS or LPS-LDL complexes on electrical resistance and LDL transport across endothelial monolayers. Confluent RAEC monolayers were washed with LPDS-DME and incubated with *E. coli* 0111:B4 LPS (2.0 ng · ml⁻¹) or with LPS complexed to LDL (5.0 μg LPS · ml⁻¹). After 3 h of incubation at 37°C, electrical resistance was measured (A). The monolayers were subsequently washed at 37°C and ¹²⁵I-LDL transport across the monolayers was determined (B) as in Fig. 1. The values shown are the mean ± 1 SD of quadruplicate determinations.

To determine if the ¹²⁵I-LPS-LDL that had crossed the RAEC monolayers was biologically active it was examined for its effect on monocyte-macrophages (8). Incubation of the transported LPS-LDL (50 ng LPS · ml⁻¹) with macrophages for 3 d resulted in a 75% reduction in the expression of scavenger receptor activity as indicated by the proteolytic degradation of MDA-LDL (1.5 ± 0.2 vs. 0.38 ± 0.03 μg ¹²⁵I-MDA-LDL degraded · 4 h⁻¹ · mg cell protein⁻¹).

We have previously shown (21) that aortic endothelial cells secrete a monocyte-specific chemotactic factor, and others have demonstrated that smooth muscle cells also secrete chemotactic factor (22, 23). As shown in a representative experiment in Fig. 3, exposure of the endothelial monolayers to transported LPS-LDL resulted in a significant increase in the

Table I. In Vitro Distribution of [³H]LPS among the Lipoprotein Classes of Rabbit Plasma*

Lipoprotein class	³ H[LPS] distribution, % of total†		
	Rabbit plasma		
	NZW	CF	WHHL
VLDL	4.5	31.0	13
LDL	7.5	30.0	68
HDL	48.5	20.5	9
d > 1.21	39.5	18.5	10

* 1 mg biosynthetically tritiated LPS ([³H]LPS), 5,500 cpm · μg⁻¹ from *S. minnesota* strain Re595 was incubated with 10 ml of plasma from fasted NZW, cholesterol-fed (CF), or WHHL rabbits for 6 h at 37°C. Samples were then fractionated by ultracentrifugation into the designated lipoprotein classes.

† Values are the means of two separate incubations and represent the percentage of total radioactivity recovered in each class.

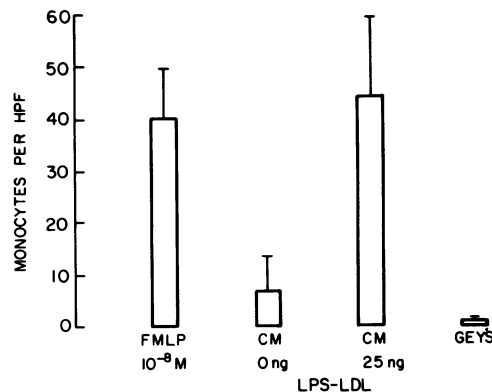


Figure 3. Increased production of monocyte chemotactic activity by endothelial cells exposed to LPS-LDL. Confluent monolayers of RAEC were exposed to transported LPS-LDL at 25 ng LPS · ml⁻¹ or incubated under the same conditions but without LPS-LDL in the growth medium for 2 d. The medium was then changed to 0.5% serum ± LPS-LDL at 25 ng · ml⁻¹. After 24 h this CM was collected, cell debris separated and discarded, and CM was tested for monocyte chemotactic activity. The addition of LDL without LPS did not induce chemotactic activity (data not shown). The chemotactic activity of FMLP or Gey's buffer containing 0.2% bovine serum albumin (Gey's) were also tested for comparison. The data represent mean ± 1 SD of triplicate measurements.

production of monocyte chemotactic factor by the endothelial cells. In a separate experiment, monocyte migration after exposure to media containing transported LPS-LDL (25, 50, or 100 ng LPS · ml⁻¹) that was incubated in the absence of cells was not different from that of buffer alone (4.9 ± 1.7 monocytes per high power field [HPF]). In contrast, in the same experiment media from RAECs incubated with transported LPS-LDL at a concentration of 25 ng LPS · ml⁻¹ resulted in a 6.5-fold increase in monocyte migration (31 ± 6 monocytes per HPF). The increase in the secretion of monocyte chemotactic activity upon exposure of the endothelial cells to transported LPS-LDL at concentrations of 25, 50, or 100 ng LPS · ml⁻¹ in nine separate experiments was 4.7 ± 3.0-fold (range, two- to ninefold increase). Checkerboard analysis indicated that the activity was chemotactic in nature and not chemokinetic (data not shown). The representative experiment in Fig. 4 demonstrates that upon exposure to LPS-LDL RASMCs also secreted substantially more monocyte chemotactic activity. In five separate experiments there was a 2.4 ± 0.6-fold increase (range, 2- to 3.2-fold increase) in the secretion of chemotactic activity by

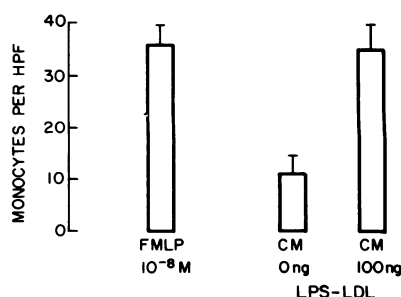


Figure 4. Increased production of monocyte chemotactic activity by smooth muscle cells exposed to LPS-LDL. Confluent RASMCs were exposed to transported LPS-LDL at 100 ng LPS · ml⁻¹ in the growth medium for 2 d. CM was collected and assayed as described in

Fig. 3. The chemotactic activity of FMLP at 10⁻⁸ M was also tested for comparison. The data represent mean ± 1 SD of triplicate measurements.

RASMCs after exposure to transported LPS-LDL at concentrations of 25, 50, or 100 ng LPS · ml⁻¹. Checkerboard analysis indicated that the activity was chemotactic and not chemokinetic (data not shown).

Discussion

Only 1–2 ng of LPS · ml⁻¹ were required to disrupt the integrity of RAEC monolayers (Figs. 1 and 2). However, LPS, at concentrations three orders of magnitude higher (5 μg LPS · ml⁻¹) when complexed to LDL, did not disrupt RAEC monolayer integrity (Fig. 2). LPS-LDL complexes were transported across RAEC monolayers at the same rate as LDL. Moreover, the transported LPS-LDL complex retained its biological activity as indicated by the ability of the complex to both suppress scavenger receptor activity on monocyte-macrophages and to stimulate RAECs and RASMCs to produce substantially more monocyte chemotactic activity after exposure to 25–100 ng LPS · ml⁻¹.

We previously reported that the association of LPS with lipoprotein appears to be remarkably stable (8). We also showed that derivatized, radioiodinated LPS bound to all lipoprotein classes, and in hyperlipidemic rabbits most of the LPS distributed to the cholesterol-rich apolipoprotein B-containing lipoproteins (8). We have now confirmed these findings with endogenously labeled LPS (Table I). Calculations based on molecular weight indicate that there are approximately an order of magnitude more HDL molecules than LDL molecules even in the plasma of cholesterol-fed, Watanabe heritable hyperlipidemic (WHHL), or LDL-infused rabbits (Table I and reference 8). However, as previously shown with derivatized, radioiodinated LPS (8) and confirmed here with endogenously labeled LPS (Table I), more than half of the LPS distributed to the cholesterol-rich apolipoprotein B-containing fractions. This suggests that LPS selectively partitions to these fractions rather than to the HDL fraction.

While LDL protects endothelial cells from the acute toxicity of LPS (Fig. 2), the resulting complexes are transported across the endothelium in a biologically active form that can initiate an inflammatory response (Figs. 3 and 4). Gerrity and colleagues (24–26), Faggiotto, Ross, and Harker (27), and Rosenfeld and colleagues (28) have demonstrated that monocyte invasion of the subendothelial space is one of the earliest events in the initiation of atherosclerosis. Schwenke and Carew (29) have recently demonstrated that LDL retention in areas later destined to be lesions precedes monocyte invasion. Since LPS-LDL appears to be transported across the endothelium at a rate similar to that for LDL it is appropriate to question if LPS-LDL might play a role in some cases in mediating the early events of atherosclerosis. In vivo experiments will be required to answer this question.

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References

1. Reidy, M. A., and D. E. Bowyer. 1977. Scanning electron microscopy: morphology of aortic endothelium following injury by endotoxin and during subsequent repair. *Atherosclerosis*. 26:319–328.
2. Gerrity, R. G., M. Richardson, B. A. Caplan, J. F. Cade, J. Hirsh, and C. J. Schwartz. 1976. Endotoxin-induced vascular endothelial injury and repair. II. Focal injury, en face morphology, (³H) thymidine uptake and circulating endothelial cells in the dog. *Exp. Mol. Pathol.* 24:59–69.
3. Harlan, J. M., L. A. Harker, M. A. Reidy, C. M. Gajdusek, S. M. Schwartz, and G. E. Striker. 1983. Lipopolysaccharide-mediated bovine endothelial cell injury in vitro. *Lab. Invest.* 48:269–274.
4. Navab, M., J. A. Berliner, A. M. Fogelman, M. E. Haberland, and P. A. Edwards. 1984. High and low density lipoproteins protect aortic endothelial cells against lipopolysaccharide toxicity. *Arteriosclerosis*. 4:528a. (Abstr.)
5. Morel, D. W., P. E. DiCorleto, and G. M. Chisolm. 1986. Modulation of endotoxin-induced endothelial cell toxicity by low density lipoprotein. *Lab. Invest.* 55:419–426.
6. Ulevitch, R. J., and A. R. Johnston. 1978. The modification of biophysical and endotoxic properties of bacterial lipopolysaccharides by serum. *J. Clin. Invest.* 63:1313–1324.
7. Ulevitch, R. J., A. R. Johnston, and D. B. Weinstein. 1979. New function for high density lipoprotein. Their participation in intravascular reactions of bacterial lipopolysaccharides. *J. Clin. Invest.* 64:1516–1524.
8. Van Lenten, B. J., A. M. Fogelman, M. E. Haberland, and P. A. Edwards. 1986. The role of lipoproteins and receptor-mediated endocytosis in the transport of bacterial lipopolysaccharide. *Proc. Natl. Acad. Sci. USA*. 83:2704–2708.
9. Navab, M., G. P. Hough, J. A. Berliner, J. A. Frank, A. M. Fogelman, M. E. Haberland, and P. A. Edwards. 1986. Rabbit beta-migrating very low density lipoprotein increases endothelial macromolecular transport without altering electrical resistance. *J. Clin. Invest.* 78:389–397.
10. Haberland, M. E., C. L. Olch, and A. M. Fogelman. 1984. Role of lysines in mediating interaction of modified low density lipoproteins with the scavenger receptor of human monocyte macrophages. *J. Biol. Chem.* 259:11305–11311.
11. Ross, R. 1971. The smooth muscle cell. II. Growth of smooth muscle in culture and formation of elastic fibers. *J. Cell Biol.* 50:172–186.
12. Colucci, W. S., T. A. Brock, M. A. Gimbrone, Jr., and R. W. Alexander. 1985. Nonlinear relationship between α-adrenergic receptor occupancy and norepinephrine-stimulated calcium flux in cultured vascular smooth muscle cells. *Mol. Pharmacol.* 27:517–524.
13. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins of human serum. *J. Clin. Invest.* 43:1345–1353.
14. McFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature (Lond.)*. 182:53.
15. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* 260:212–221.
16. Schechter, I., A. M. Fogelman, M. E. Haberland, J. Seager, M. Hokom, and P. A. Edwards. 1981. The metabolism of native and malondialdehyde-altered low density lipoproteins by human monocyte-macrophages. *J. Lipid Res.* 22:63–71.
17. Ulevitch, R. J. 1978. The preparation and characterization of a radioiodinated bacterial lipopolysaccharide. *Immunochemistry*. 15:157–164.
18. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoproteins by cultured fibroblasts. *J. Biol. Chem.* 249:5153–5162.
19. Falk, W., R. M. Goodwin, Jr., and E. J. Leonard. 1980. A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J. Immunol. Methods*. 33:239–247.

20. Lowry, O. H., M. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
21. Berliner, J. A., M. Territo, L. Almada, A. Carter, E. Shafonsky, and A. M. Fogelman. 1985. Monocyte chemotactic factor produced by large vessel endothelial cells in vitro. *Arteriosclerosis.* 6:254-258.
22. Mazzone, T., M. Jensen, and A. Chait. 1983. Human arterial wall cells secrete factors that are chemotactic for monocytes. *Proc. Natl. Acad. Sci. USA.* 80:5094-5097.
23. Valente, A. J., S. R. Fowler, E. A. Sprague, J. L. Kelley, C. A. Suervlan, and C. J. Schwartz. 1984. Initial characterization of a peripheral blood mononuclear cell chemoattractant derived from cultured arterial smooth muscle cells. *Am. J. Pathol.* 117:409-417.
24. Gerrity, R. G. 1981. The role of the monocyte in atherogenesis. I. Transition of blood-borne monocytes into foam cells in fatty lesions. *Am. J. Pathol.* 103:181-190.
25. Gerrity, R. G. 1981. The role of the monocyte in atherogenesis. II. Migration of foam cells from atherosclerotic lesions. *Am. J. Pathol.* 103:191-200.
26. Gerrity, R. G., J. A. Goss, and L. Soby. 1985. Control of monocyte recruitment by chemotactic factor(s) in lesion-prone areas of swine aorta. *Arteriosclerosis.* 5:55-66.
27. Faggiotto, A., R. Ross, and L. Harker. 1984. Studies of hypercholesterolemia in the nonhuman primate. I. Changes that lead to fatty streak formation. *Arteriosclerosis.* 4:323-340.
28. Rosenfeld, M. E., T. Tsukada, A. M. Gown, and R. Ross. 1987. Fatty streak initiation in Watanabe heritable hyperlipemic and comparably hypercholesterolemic fat-fed rabbits. *Arteriosclerosis.* 7:9-23.
29. Schwenke, D. C., and T. E. Carew. 1986. LDL content and rate of LDL degradation near aortic branch orifices increase with cholesterol feeding. *Arteriosclerosis.* 6:256a. (Abstr.)