Regulation of Macrophage ApoE Expression and Processing by Extracellular Matrix*

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Macrophage-derived apoE in the vessel wall has important effects on atherogenesis in vivo, making it important to understand factors that regulate its expression. Vessel wall macrophages are embedded in an extracellular matrix produced largely by arterial smooth muscle cells and endothelial cells. In this series of studies, we evaluated the influence of extracellular matrix on macrophage apoE expression. Subendothelial matrix, fibronectin, or collagen I stimulated macrophage apoE gene expression and apoE synthesis. Adhesion of macrophages to a polylysine substrate had no effect. An increase in apoE synthesis after plating on fibronectin could be observed by 2 h and was inhibited by blocking antibodies to the α5β1 integrin receptor for fibronectin. Fibronectin also regulated the post-translational processing of newly synthesized macrophage apoE by inhibiting its degradation. The increment in apoE resulting from suppressed degradation was retained in the cell-fibronectin monolayer in a pool that was resistant to release by exogenous high density lipoprotein subtraction 3. These observations establish a new pathway for the regulation of macrophage apoE expression in the vessel wall. The composition of the extracellular matrix changes after vessel wall injury and in response to locally produced cytokines and growth factors. The evolving composition of this matrix will, therefore, be important for regulating apoE expression and processing by vessel wall macrophages.

In the vessel wall, apoE is found in association with macrophages and the extracellular matrix (1, 2). Macrophages are the major source of newly synthesized apoE in the vessel wall, and a great deal of information is available regarding the influence of macrophage-derived apoE on vessel wall pathology (3, 4). Studies using bone marrow transplantation approaches, to specifically modulate macrophage-derived apoE production in the vessel wall, have shown an important influence on atherogenesis in multiple models of atherosclerosis-prone mice. In most of these studies, macrophage-derived apoE has been found to be atheroprotective (3–6). Multiple mechanisms can be considered for the atheroprotection afforded by macrophage-derived apoE. ApoE particles secreted by macrophages have been shown to have antioxidant properties and have been shown to influence the growth and motility of endothelial cells and arterial smooth muscle cells (3, 4, 7). Macrophage-derived apoE also transduces sterol efflux from macrophages and may, thereby, limit foam cell formation in the vessel wall (3, 4, 8–11).

Factors that regulate macrophage apoE expression in the vessel wall, therefore, will have important effects on vessel wall disease. Cellular sterol content regulates macrophage apoE gene expression via liver X receptor (LXR) elements (12–14). Bacterial endotoxin and cytokines have also been shown to influence macrophage apoE expression (15, 16). In the vessel wall, macrophages are embedded in an extracellular matrix, produced predominantly by endothelial cells and arterial smooth muscle cells. The composition of this matrix evolves after injury to the vessel wall, and this changing composition may have important effects on the behavior of vessel wall cells (17–19). For example, the composition of the extracellular matrix has been shown to modulate the phenotype of arterial smooth muscle cells. In the current studies, we explored the hypothesis that components of the extracellular matrix modulate macrophage apoE expression. This hypothesis was based on considerations regarding the importance of macrophage-derived apoE in the vessel wall for atheroprotection and the emerging role of matrix composition for modulating cellular behavior in the vessel wall. We found that the subendothelial matrix as well as specific components found in this matrix can stimulate macrophage apoE gene expression. Furthermore, we found that fibronectin (an abundant ECM1 component with modified expression after vessel wall injury) can significantly modulate the post-translational handling of apoE by the macrophage.

EXPERIMENTAL PROCEDURES

Materials—The goat-derived anti-human apoE polyclonal antibody was purchased from International Immunology Corp. (Murrieta, CA). The mouse monoclonal anti-human integrin β1 (monoclonal antibody 2253) and the rat anti-mouse integrin α5β1 (monoclonal antibody 1984) were obtained from Chemicon International Inc. (Temecula, CA). A monoclonal anti-human cardiac myoglobin antibody was purchased from American Research Products Inc. (Belmont, MA). Cell culture dishes coated with fibronectin, collagen I, or polylysine were purchased from Becton Dickinson (Bedford, MA). SuperFect transfection reagent was purchased from Qiagen (Chatsworth, CA). Assay kits for luciferase, β-galactosidase, and plasmids pGL3 and pSV β-galactosidase were purchased from Promega (Madison, WI). All tissue culture reagents were obtained from Invitrogen (Gaithersburg, MD). [35S]Methionine was purchased from Amersham Biosciences (Arlington Heights, IL). HDL3 (d = 1.125–1.210 g/ml) was isolated from human plasma as previously described (8), and was free of apoE by SDS-PAGE. All other

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The abbreviations used are: ECM, extracellular matrix; SEM, subendothelial matrix; HDL3, high density lipoprotein subfraction 3; FMA, phorbol 12-myristate 13-acetate; FN, fibronectin; RT, reverse transcription.
Materials were from previously described sources (20, 21). Cells and Subendothelial Matrix—Human monocytes were isolated by elutriation, were ~95% pure, and were grown as previously described (15, 22). The cells were allowed to differentiate for 72 h in DMEM containing 20% FBS and 10% pooled human AB serum before being used for experiments. THP-1 monocytes (Life Technologies, Inc.) were previously described (12). J774E cells were maintained as a suspension culture in RPMI 1640 containing 10% FBS and $2 \times 10^{-3} \text{M } \beta$-mercaptoethanol. For experiments, these cells were treated with 10 or 20 ng/ml PMA to induce macrophage differentiation. RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS. Mouse peritoneal macrophages were collected from CD1 mice (Charles River Laboratories International, Inc.) as previously described (12). J774E cells were injected i.p. into BALB/c mice that were maintained as a suspension culture in RPMI 1640 containing 10% FBS and $2 \times 10^{-3} \text{M } \beta$-mercaptoethanol. For experiments, these cells were treated with 10 or 20 ng/ml PMA to induce macrophage differentiation. RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS. Mouse peritoneal macrophages were collected from CD1 mice (Charles River Laboratories International, Inc.) as previously described (12). J774E cells were then cultured in a suspension culture in RPMI 1640 containing 10% FBS and $2 \times 10^{-3} \text{M } \beta$-mercaptoethanol. For experiments, these cells were treated with 10 or 20 ng/ml PMA to induce macrophage differentiation. 

SEM-CON Apo E

$9.2 \pm 0.7$ $5.8 \pm 0.9$ $P < 0.01$

FIG. 1. Subendothelial matrix stimulates macrophage apoE synthesis. Human THP-1 monocytes were grown on uncoated dishes (CON) or on dishes coated with a matrix synthesized by endothelial cells (SEM). PMA (20 ng/ml) was included to induce macrophage differentiation. After 46 h, cells were labeled with $^{38} \text{S}$methionine and harvested 2 h later for isolation of cellular apoE. The values shown are the mean ± S.D. from the triplicate lanes shown. The difference in cellular apoE between cells grown in uncoated versus SEM-coated dishes is significant at $p < 0.01$.

β-actin primer pair (5′ primer, GCC CCA GAG CAA GAC AGG TA; 3′ primer, GGC CTA ATC GTA CTC CTG CT) produced a 924-bp product. Different amounts of input RNA, cycling temperatures, and cycle number were evaluated to assure a linear response of the apoE and β-actin signals under the conditions of our experiments. The PCR products were resolved on agarose gels and stained with ethidium bromide. The image was captured using a documentation system (UVP, Upland, CA) and quantitated using ImageQuant software.

To coat plates with subendothelial matrix, bovine aortic endothelial cells were isolated by collagenase treatment of bovine aorta and were grown to confluence in 10% FBS in RPMI 1640. At confluence, the cells were treated with 0.1% Triton X-100 and 20 mM NH$_4$OH in PBS for 7 min at room temperature, followed by four washes with PBS. The remaining matrix was washed once with 1% BSA. This procedure has been shown to completely remove the cell monolayer and leave behind matrix secreted by endothelial cells (23).

Macrophage ApoE Synthesis and Stability—The synthetic rate for apoE was monitored by the quantitative immunoprecipitation of radiolabeled apoE as previously described (21). After a pulse-labeling incubation with $^{38} \text{S}$methionine, macrophage monolayers were washed twice with PBS, incubated with 100 μl of 2% SDS, and heated at 95°C for 5 min. Thereafter, 0.9 ml of lysis buffer (10 mM Na$_2$PO$_4$, 15 mM NaOH, 10 mM methionine, 1% Triton X-100, and 1% deoxycholate, pH 7.4) was added to each dish. The cells were incubated at room temperature for 2 h, and the cell lysate was sheared with a 25-gauge needle and spun at 10,000 rpm to remove particulate debris. The supernatant was utilized for quantitative immunoprecipitation by methods previously described in detail (21). Immunoprecipitations were begun using equal numbers of trichloroacetic acid-precipitable counts. Therefore, changes in apoE synthesis are already corrected for any changes in total protein synthesis and for any variability in the number of cells attached to the different matrices. There were no systematic differences in trichloroacetic acid-precipitable counts between cells plated on control or matrix-coated substrates. The post-translational processing of the newly synthesized cellular apoE protein was evaluated using a pulse-chase experimental format. Cells were pulse-labeled for 45 min in 200 μM $^{38} \text{S}$methionine and then chased with medium containing 500 μM unlabeled methionine for the times indicated in the figures. ApoE remaining in cell lysates, or apoE secreted into the medium, was recovered by quantitative immunoprecipitation as described above. As above, radioactivity in apoE was corrected for any changes in total cellular protein synthesis. For all experiments, the immunoprecipitated apoE was resolved on SDS-PAGE. The radioactive signal present in apoE was detected and quantitated using an Amersham Biosciences PhosphorImager with ImageQuant NT software. The results are expressed as scanning cpm. Appropriate experimental controls were included on each SDS-PAGE gel to allow direct comparison of the effect of incubation conditions on apoE synthesis.

Quantitation of ApoE mRNA—For Northern analysis, total cellular RNA was isolated after solubilizing cells in guanidine isothiocyanate, followed by sedimentation of the extract through cesium chloride. Formaldehyde-treated RNA samples were fractionated by electrophoresis in 1% agarose and transferred to nylon membranes as previously described (16). The membranes were hybridized with a labeled apoE cDNA probe as previously described (16). The radiolabeled apoE cDNA probe was prepared by random prime synthesis utilizing the full-length human apoE cDNA as a template. Each reaction was carried out using 50 ng of cDNA with a minimum of $1 \times 10^6$ cpm. To provide an internal standard, the nylon membranes were stripped and reprobed with a labeled 0.7-kb PST1 fragment of the cDNA for β-actin. ApoE and β-actin signals were detected and quantitated using the Amersham Biosciences PhosphorImager and ImageQuant NT software. Results were expressed as apoE/β-actin ratios from each sample to correct for loading or any variability in the number of cells attached to the different matrices.

For RT-PCR, cytoplasmic RNA was isolated from mouse peritoneal macrophages grown on uncoated or fibronectin-coated substrates for 48 h using the RNeasy Mini Kit (Qiagen). RT-PCR was performed using 10 ng of RNA and the Calypro RT-PCR kit from DNAmp Ltd. The apoE primer pair (5′ primer, AGG ATC TAC GCC ACA GAC TC; 3′ primer, GCC GAT GCA TGT CTT CCA CT) produced a 500-bp product.

In the next series of experiments, we investigated the effect of specific components of subendothelial matrix on the macro-
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PMA (ng/ml) 10 20
ApoE/β-Actin 9.8 15.2

![SEM Control](image)

Fig. 2. Subendothelial matrix stimulates macrophage apoE mRNA abundance. Human THP-1 monocyte cells were grown on uncoated dishes (Control) or on a matrix synthesized by endothelial cells (SEM). PMA (10 or 20 ng/ml) was included to induce macrophage differentiation as indicated. After 24 h, cells were harvested for measurement of apoE mRNA abundance. The values shown represent the apoE signal after correction for the β-actin signal (ApoE/β-Actin) and are the averages from duplicate lanes shown.

an internal control for transfection efficiency. After the incubations described in the legend of Table I, luciferase activity was measured and, after correction for β-galactosidase activity, was 4- to 5-fold higher in cells cultured on fibronectin or collagen I.

In considering mechanisms for how ECM could stimulate apoE expression in macrophages, we considered the observations that components of ECM have been shown to bind to specific cell surface receptors and to, thereby, stimulate generation of second messengers (28–30). Such a mechanism would likely lead to an early response, and therefore, we evaluated the level of apoE synthesis in mouse peritoneal macrophages after a short (2 h) incubation on matrix-coated or uncoated substrates. As shown in Fig. 4, the response to fibronectin after a 2-h incubation was −2-fold. The level of apoE expression after a 2-h incubation on collagen I was less consistent at 2 h over multiple experiments compared with the fibronectin response. In evaluation of multiple time points for the fibronectin response in mouse peritoneal macrophages, the 2-h increase in apoE synthesis varied between 1.4- and 2.0-fold, whereas the response at 24 or 48 h varied between 2- and 3-fold. Because of the consistent and significant increase observed in apoE synthesis after 2 h on fibronectin, we focused on the fibronectin response and evaluated the role of the α5β1 integrin receptor in mediating the macrophage apoE response to fibronectin. These experiments are shown in Fig. 5. We utilized two different blocking antibodies: one specific for the integrin β1 subunit, and the second for the α5β1 heterodimer. Each of these antibodies significantly reduced the level of apoE synthesis in mouse peritoneal macrophages during a 2-h incubation on fibronectin. The blocking antibodies had no effect on apoE synthesis in cells cultured on uncoated dishes (Fig. 5) or on collagen I (not shown). Inclusion of an irrelevant antibody to cardiac myoglobin did not reduce apoE synthesis in mouse peritoneal macrophages cultured on fibronectin (not shown).

The above experiments demonstrated that extracellular matrix can stimulate apoE gene expression, resulting in increased synthesis of apoE. However, because a substantial portion of newly synthesized macrophage apoE is rapidly degraded (prior to secretion), factors that regulate the post-translational processing of apoE can also significantly alter its net expression in the macrophage (21, 31–33). Therefore, we next investigated whether FN could modulate apoE expression at such a post-translational locus using a pulse-chase experimental format (Fig. 6). Mouse peritoneal macrophages were plated on uncoated or fibronectin-coated substrates for 48 h prior to pulse labeling with [35S]methionine for 45 min. A chase incubation was then started and is designated as “0 min” in the figures. Over the subsequent 120 min of chase, more apoE was released from the cells cultured on fibronectin-coated plates (panel A); however, the magnitude of this increase was somewhat smaller than the increment in synthesis (shown in panel B at 0 min). This reduced release of apoE (compared with synthesis) could be due to increased degradation of newly synthesized apoE by cells in the presence of FN or increased retention of newly synthesized apoE in the cell-fibronectin monolayer. Measuring the disappearance rate of cellular apoE (panel B) provided information regarding this issue. At the start of the chase incubation, there was an approximate 2-fold increase in cellular apoE on FN-coated plates (reflecting the increased apoE synthesis during the pulse incubation). Over the subsequent 120 min, apoE levels fell in the cell monolayers plated on both fibronectin-coated and uncoated substrates. However, the half-life of apoE disappearance was longer in the fibronectin monolayer (87 min) compared with the monolayer plated on an uncoated surface (36 min). These results are consistent with...
increased retention of apoE in the cell-FN monolayer as contributing to the smaller increment in apoE secretion compared with synthesis. However, the increased retention of apoE in the FN-cell monolayer raised an additional question. Because newly synthesized apoE is removed from the cell layer by either secretion or rapid degradation, the increased retention of apoE in the FN-cell monolayer could also reflect suppressed degradation of newly synthesized apoE. Evaluating a potential change in degradation in mouse peritoneal macrophages is complicated by the different rates of apoE synthesis in cells on the different substrates. To eliminate this potential confounding influence, we utilized a model in which apoE synthetic rates would be identical on uncoated and fibronectin-coated plates.

The J774 macrophage cell line, which does not express its endogenous apoE gene, was transfected to stably express a human apoE3 cDNA under the control of a constitutively expressed cytomegalovirus promoter (J774E/H11001 cells). These cells were plated on uncoated or fibronectin-coated substrates for 48 h prior to a pulse-chase analysis conducted exactly as described for mouse peritoneal macrophages.

For all three cell types, the increase in apoE synthesis on fibronectin or collagen is significant at \( p < 0.01 \), compared with apoE synthesis from cells plated on uncoated or polylysine-coated surfaces.

### FIG. 3

Fibronectin and collagen I stimulate apoE synthesis in THP-1, human monocyte-derived, and mouse peritoneal macrophages. THP-1 cells (A), human monocyte-derived macrophages (B), or mouse peritoneal macrophages (C) were plated on uncoated surfaces (CON) or on surfaces coated with fibronectin (FN), collagen I (CNI), or polylysine (Poly-Ly) as indicated. For THP-1 cells, 20 ng/ml PMA was included to induce macrophage differentiation. After 48 h, cells were pulse-labeled with \(^{35}\)S-methionine for 45 min. At that time, cell monolayers were harvested for isolation and measurement of apoE. The values shown represent the mean ± S.D. from triplicate wells of cells.

### TABLE I

| Substrate       | RLUa                        |
|-----------------|-----------------------------|
| CON             | 1.7 ± 0.3                   |
| Fibronectin     | 9.8 ± 0.5                   |
| Collagen I      | 9.3 ± 0.7                   |

a RLU, relative luciferase units.

### FIG. 4

Short-term exposure to matrix components increases apoE synthesis. Mouse peritoneal macrophages were plated on fibronectin (FN), collagen I (CNI), or uncoated (CON) surfaces for 2 h and then pulse-labeled for 45 min with \(^{35}\)S-methionine. Cells were then harvested for isolation and measurement of apoE. The increase in apoE synthesis on fibronectin compared with uncoated surface is significant at \( p < 0.01 \). The increase in apoE synthesis on collagen I compared with uncoated surface is significant at \( p < 0.05 \). Values shown are mean ± S.D. from triplicate wells of cells.

For all three cell lines, the increase in apoE synthesis on fibronectin or collagen is significant at \( p < 0.01 \), compared with apoE synthesis from cells plated on uncoated or polylysine-coated surfaces.
in this cell model synthesis rates for apoE at the end of the pulse incubation ("0 min" of chase) are identical for the cells on the two different surfaces (panel B). In panel A, it can be seen that the secretion of apoE from J774E\(^{+}\) cells is similar on the two substrates through 90 min of chase. Examination of the rate of disappearance of apoE from the cell monolayer (in panel B) confirms its slower disappearance rate in the presence of fibronectin (\(t_{1/2} = 89\) min on fibronectin compared with 56 min on uncoated surface). The results of these experiments indicate that the increment in apoE synthesis produced by either blocking antibody on fibronectin-coated surfaces is significant at \(p < 0.01\). NA, no antibody.

**DISCUSSION**

The data in this report reveal a new pathway for regulation of macrophage apoE gene expression that is highly relevant to the vessel wall. Subendothelial matrix stimulates apoE gene expression and apoE synthesis in macrophages. This stimulation can be accounted for by the presence of collagen I or...
TABLE II
Suppressed degradation of newly synthesized apoE by fibronectin

|             | ApoE in cell lystate | ApoE released into the medium | ApoE degraded |
|-------------|----------------------|-----------------------------|---------------|
| FN-coated   |                      | 45.6                        | 15.9          | 38.5          |
| Uncoated    |                      | 33.9                        | 14.7          | 51.4          |

Fig. 8. Release of apoE from the cell monolayer by HDL3.

J774 E- cells were pulse-labeled and chased for 90 min as described in the legend to Fig. 7. The scanning cpm measured in cell lystate and medium at 90 min were divided by the scanning cpm present in cell lystate at 0 min to yield the percentage released into the medium, or the percentage retained in the cell lystate. These values were subtracted from 100% to estimate degradation of newly synthesized apoE at 90 min.

Increased apoE synthesis can be detected by 2 h after exposure of macrophages to fibronectin. Furthermore, this rapid response can be inhibited by blocking antibodies to α5β1, the major cell surface integrin receptor for fibronectin. Cell surface integrins are a diverse group of heterodimeric transmembrane proteins that mediate cell adhesion (28–30). However, beyond this, integrins also transduce signals that regulate organization of the cellular cytoskeleton and gene transcription (28–30). This signaling may be cell-specific and is subject to affinity modulation by conformational changes in the integrins. It is interesting that such affinity modulation has been reported after stimulation of leukocytes with phorbol ester, and this may account for the enhanced response of apoE mRNA levels to FMLP that we observed in the presence of higher concentrations of PMA (Fig. 2). It is also of interest that inclusion of the β1 integrin-blocking antibody reduced apoE synthesis in the presence of fibronectin to a level below that observed on control plates. This could reflect an effect of fibronectin on apoE synthesis mediated through other cell surface receptors or by mediating enhanced cell anchorage.

In addition to transcriptional regulation, it is also apparent that fibronectin can modulate the net production of apoE by macrophages at a post-translational level. Because newly synthesized macrophage apoE is both secreted and degraded (21, 31–33), the reduced rates of disappearance from cell lystate we observed in mouse peritoneal macrophages grown on fibronectin are consistent with either lower rates of secretion or suppressed degradation. In mouse peritoneal macrophages the increment in apoE secretion did appear to be lower than that expected for the increase in synthesis, supporting a reduction in secretion as contributing to the reduced rate of disappearance from the cell monolayer. Evaluating any additional contribution of suppressed degradation in mouse peritoneal macrophages is complicated by different synthesis and secretion rates in the presence of FN. However, this issue was further clarified in a cell model in which synthesis of apoE was identical on uncoated and fibronectin-coated plates. The results of this evaluation indicated that the persistence of apoE in the cell layer in the presence of fibronectin was also due to suppressed degradation of newly synthesized apoE. Furthermore, the results in Fig. 7 rule out the possibility that the increased apoE present in the cell-matrix monolayer on FN-coated plates is solely due to re-uptake of secreted apoE, because this would be expected to reduce the amount of apoE present in the medium. In fact, the observation that approximately equal amounts of apoE are secreted from J774E- grown on FN or uncoated substrate, indicated that the increment of apoE in the FN associated cell monolayer was not derived from secreted apoE or from a pool of cellular apoE destined for secretion but most likely from a pool destined for degradation.

We have previously shown that the newly synthesized apoE retained by macrophages is present in an intracellular as well as a cell surface location (22). We have also shown that apoE present on the cell surface recycles to the intracellular compartment, and that a portion of this apoE is degraded before it can be released (22). The release of both cell surface and intracellular apoE can be facilitated by the addition of extracellular HDL3 (21, 22, 31–33). When we utilized HDL3 to mobilize cell surface (4 ºC) and total cellular (37 ºC) apoE, less apoE was released from the cells on fibronectin-coated plates, despite the fact that more apoE was present in this monolayer. This

*Note: The image contains tables and figures that are not transcribed into text format.*
suggested that the apoE sequestered in the monolayer on fibronectin-coated plates was trapped in a pool not present in cells grown on uncoated surfaces; i.e. trapped in the fibronectin matrix. Because of our previous observations regarding the recycling and degradation of cell surface apoE in the macrophage (22), we believe the most likely explanation for suppressed apoE degradation on fibronectin-coated plates is the interruption of apoE recycling by the sequestration of cell surface apoE by extracellular fibronectin, thereby sparing it from re-internalization and subsequent intracellular degradation. This is the complete opposite of what occurs when endogenously synthesized apoE is bound to an endogenously synthesized pericellular proteoglycan matrix. In this circumstance, the recycling and degradation of apoE is facilitated. An alternative mechanism for suppressed degradation in the presence of fibronectin could be integrin-mediated signaling and regulation of intracellular degradation pathways. However, this explanation is somewhat less likely in that it would not completely explain the failure to see a proportionally increased secretion of apoE as a result of its suppressed degradation. Furthermore, in preliminary experiments, inhibitors of integrin-mediated signal transduction were not able to suppress the effects of fibronectin on apoE degradation (not shown).

ApoE produced by macrophages in the vessel wall has important implications for vessel wall physiology. In most in vivo models, macrophage-derived apoE has been found to be athero-protective (3–6). Macrophage-derived apoE has been shown to have antioxidant properties, can influence the behavior of multiple cell types found in the vessel wall, and can modulate sterol efflux from cells in the vessel wall (3–6, 8–11). ApoE interaction with matrix components may also have important implications for retention of lipoproteins or growth factors in the vessel wall (23). All of these considerations make it important to understand factors that modulate apoE expression in macrophages and that are relevant to vessel wall biology. It has been well established that apoE interacts with proteoglycans in the ECM, and it has been strongly associated with biglycan (1, 34). The apoE interaction with biglycan is at least bifunctional, involving an electrostatic association with charged groups on glycosaminoglycans and a separate ionic interaction with the biglycan core protein (35). Although it has been generally appreciated that proteoglycans in the extracellular matrix can bind apoE, including cell-derived apoE, it is becoming apparent that other components of ECM can be involved. Huang et al. (36) have shown that apoE has high avidity for laminin, probably via protein-protein interactions. The composition of the vessel wall ECM changes after injury and evolves with progression of the atherosclerotic plaque (17, 18, 25). Regions of increased synthesis of type I collagen and fibronectin can be demonstrated; local production of cytokines or growth factors modulate production of these ECM components by arterial smooth muscle cells and endothelial cells (17, 18, 25, 37). For example, transforming growth factor β increases collagen I synthesis, and lysophosphatidic acid (a phospholipid growth factor released by platelets and contained in lipoproteins) stimulates fibronectin synthesis. Furthermore, vessel wall injury not only promotes the synthesis of fibronectin, it also leads to the appearance of fibronectin isoforms that are usually absent from vessel wall (25). Hypertension and angiotensin II also modulate fibronectin synthesis and isoform distribution in the arterial wall (25). Our results establish, therefore, that the changing composition of ECM (in response to vessel wall injury, cytokines, or growth factors) will have importance for regulating the expression and processing of newly synthesized apoE by vessel wall macrophages.

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