Tumor Necrosis Factor-α Modulates Monocyte/Macrophage Apoprotein E Gene Expression

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Abstract

apo E has been shown to modulate cholesterol balance in arterial wall cells. Production of apo E by macrophages in atherosclerotic plaques could thereby influence the development of the plaque lesion. Cytokines, including TNFα, have been identified in human lesions, therefore, we undertook a series of studies to evaluate the effect of TNFα on monocyte/macrophage apo E production. The addition of TNFα to freshly isolated human monocytes led to a four- to fivefold increase of apo E mRNA abundance. The addition of TNFα to fully differentiated macrophages either had no effect or modestly inhibited apo E mRNA expression. THP1 human monocytic cells also responded to TNFα in a phenotype-specific manner. Treatment of these cells with TNFα produced a dose- and time-dependent increase in apo E mRNA. This increase was reflected in apo E synthesis and was associated with inhibition of DNA synthesis, and with induction of c-fos and ICAM-1 gene expression. Cell-permanent analogues of ceramide did not reproduce TNFα effect on apo E, but antagonists of protein kinase C did inhibit its effect. TNFα induction of apo E mRNA abundance was associated with stimulation of apo E promoter-dependent gene transcription. In summary, TNFα stimulates apo E gene transcription, mRNA abundance, and protein synthesis in the monocyte/macrophage in a phenotype-specific manner. Such regulation could significantly modify the amount of apo E present in vessel wall lesions. (J. Clin. Invest. 1995. 96:915–922.) Key words: cytokines • atherosclerosis • macrophage activation • protein kinase C • ceramide

Introduction

apo E is an important structural and functional constituent of plasma lipoproteins, including VLDL, HDL, and chylomicron remnants (1). Although apo E is primarily synthesized by the liver, significant amounts are also produced by a variety of peripheral tissues and cells (2), including macrophages, where its synthesis is regulated by the cholesterol content of the cells (3, 4). Histochemical studies have indicated that macrophages are the major source of locally derived apo E in developing vessel wall atheromatous lesions (5). Local production of apoE in the vessel wall could impact on proximate cellular cholesterol flux and modify the local availability of bioactive molecules such as growth factors (1). Furthermore, endogenous production of apo E has been shown to influence macrophage cholesterol flux by regulating the rate of cellular cholesterol clearance to HDL (6). Thus, modulation of macrophage apo E secretion in the vessel wall could significantly impact atheroma progression or regression.

Regulation of macrophage apo E expression is complex: it is regulated not only by cholesterol but also by activators of protein kinase C and by endotoxin (7–9). TGFβ has been demonstrated to induce macrophage apo E expression (10). On the other hand, the monocyte colony stimulating factor (M-CSF) has been shown, in separate reports, both to inhibit and augment macrophage apo E expression (10, 11). A potential regulatory role for other cytokines, for example, TNFα, has been even less clear (10, 12).

Local production of TNFα is an important component of the vessel wall response to injury. TNFα positivity has been found in the cytoplasm of macrophages, and in the cytoplasm and attached to cell membranes of smooth muscle cells and endothelial cells of the human atheroma (13, 14). These observations suggest a potential for TNFα to modulate the evolution of the atherosclerotic plaque. Further, such modulation would be likely to occur via effects on monocyte/macrophage cells because these are primary targets of TNFα action. In the present study, we investigated the effect of TNFα on apoE expression in the human monocyte/macrophage. We examined phenotype-dependent effects of TNFα on apo E gene expression and compared these with its effects on the expression of other monocyte/macrophage genes (c-fos and intercellular adhesion molecule [ICAM]-1) and cell cycle traverse. We also investigated potential roles for ceramide second messengers and protein kinase C (pKc) activation for transducing the observed effects of TNFα on apo E.

Methods

Materials. [35S]Methionine (10 Ci/mmol), [32P]dCTP (800 Ci/mmol), and [3H]thymidine (5 Ci/mmol) were purchased from Amer sham Corp. (Arlington Heights, IL). Human recombinant TNFα was purchased from Genzyme, Corp. (Boston, MA). C2-ceramide and H7 were purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). Endotoxin-free BSA came from Calbiochem Corp. (La Jolla, CA).

1. Abbreviations used in this paper: ICAM, intercellular adhesion molecule; M-CSF, monocyte colony stimulating factor; NFκB, nuclear factor-κB; pKc, protein kinase C.
Jolla, CA). All other materials were obtained from previously identified sources (6, 9, 15, 16).

Cell culture. Freshly isolated human monocytes were purified by elutriation. The cell population used for experiments was > 95% monocytic, as determined by differential counts of Wright stained smears. These cells were incubated using conditions described in the figure legends. For experiments using fully differentiated human monocyte-derived macrophages, cells were allowed to differentiate in serum-containing medium for 7 d before the start of the incubation with TNFa.

The THP1 human monocytic cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD), and cells were grown in culture flasks (Falcon Plastics, Cockeysville, MD) in RPMI-1640 medium containing 10% heat-inactivated FBS as described (15). For experiments, all cells were washed twice with serum-free RPMI-1640 and were placed in RPMI-1640 medium supplemented with 0.2% endotoxin-free BSA unless otherwise indicated.

Immunoprecipitation of apo E. THP1 cells were pulse-labeled with [35S]methionine, as described in detail previously (16). Briefly, cells were plated at a density of 5 x 10^5 cells in a 60-mm dish in triplicate. 5 ng/ml of TNFa was added, and the cells were incubated for 72 h at 37°C. After incubation, adherent cells and cells in suspension were washed twice with methionine-free RPMI-1640 and then incubated for 2 h in 1 ml of methionine-free RPMI-1640 medium supplemented with 100 μCi/ml of [35S]methionine supplemented with 10 μM unlabeled t-methionine. After incubation, the medium was collected after centrifugation to remove cells. Adherent cells and cells in suspension were washed twice in ice-cold phosphate-buffered saline and lysed in 2.0% SDS at 95°C for 3 min. The lysate was then diluted with lysis buffer (10 mM Na2HPO4, 15 mM NaCl, 10 mM L-methionine, 1% Triton X-100, 1% deoxycholate, pH 7.4) to final concentration of 0.2% SDS. Cell extracts and culture media were analyzed by immunoprecipitation followed by 10% SDS-PAGE and fluorography as previously described (16). 4 x 10^4 TCA-precipitable counts/min were immunoprecipitated from each cell lysate sample, and 4 x 10^3 counts/min were immunoprecipitated from each medium sample. Incorporation of labeled methionine into apo E protein in THP1 cells was linear over the assay period. For quantitation, apo E bands were excised from the gel and digested in 30% H2O2 for scintillation counting (16). Radioactivity in the apo E shown was normalized for rate of total protein synthesis and secretion, which was not significantly different between groups.

RNA isolation and Northern hybridization. Cells were seeded at a density of 10 x 10^6 cells in a 100-mm dish. To isolate total cellular RNA, washed cells were solubilized in guanidine isothiocyanate as described, followed by sedimentation of the extract through CsCl (9). For some experiments, poly (A)+ RNA was purified after passing total RNA through silanized oligo (dT) columns. For Northern analysis, formaldehyde-treated RNA samples were fractionated by electrophoresis in 1.0% agarose, transferred to Nytran (Schleicher & Schuell, Inc., Keene, NH), and hybridized with a labeled apo E cDNA probe as previously described (9). Radiolabeled apo E cDNA probe was prepared by random-primed synthesis using pHE4A, which is a 1.1-kb AatII-Hinf I fragment that encompasses the entire protein coding region of the human apo E cDNA subcloned into pUC (9). Each reaction was carried out using 50 ng of cDNA with a minimum of 1 x 10^7 cpn. To provide an internal control, Nytran membranes were stripped according to the manufacturer’s instructions and reprobed with a labeled 0.7-kb PatI fragment of the cDNA for beta-actin. The c-fos probe, which was a 1.0-kb PatI subfragment from the fos cDNA and the ICAM-1 probe, which was a 1.4-kb BglII-SalI subfragment from the ICAM-1 cDNA, was labeled using the same method.

Measurement of DNA synthesis. Rates of DNA synthesis were assessed by determining [3H]thymidine incorporation into TCA precipitable material (17). After cultures were treated with the indicated amount of TNFa for 24 h, cells were pulsed with 2.5 μCi/ml [3H]thymidine for 2 h and then washed with RPMI-1640 five times. This was followed by five washes with ice-cold 5% TCA. Cells were then extracted in 1 ml of 0.25 N NaOH for scintillation counting. Protein was assayed using Lowry’s method (18).

Cell transfection and measurement of luciferase activity. An apo E-luciferase chimeric construct, ~2300/ +24 apo E pGL2, was made using standard recombinant techniques (19). The 5.1-aAtll fragment of the apo E gene was cloned into the polylinker region of pGL-2 Basic (Promega Corp. Madison, WI). THP1 cells were transfected using a modified dextran method (20). 1 x 10^7 cells were transfected with 2 μg of ~2300/ +24 apo E pGL2 plasmid in 1 ml STBS buffer (25 mM Tris-Cl, 137 mM NaCl, 5 mM KCl, 0.6 mM NaHPO4, 0.7 mM CaCl2, 0.5 mM MgCl2) with 100 μg DEAE dextran for 90 min and shocked with 10% DMSO for 5 min. After transfection, the cells were incubated in RPMI medium with 10% FBS overnight. At that time, all transfected cells were mixed in a single pool prior to being aliquoted to experimental treatments, to eliminate differential transfection efficiency as an experimental variable. The medium was then changed to RPMI with 0.2% endotoxin-free BSA, and 10 ng/ml of TNFa-α was added. Before harvesting, the cells were left at room temperature for 20 min; the cells were then lysed, and luciferase activity was measured using a luciferase assay kit and a luminometer (Enhanced Luciferase Assay Kit; Luminometer Monolight 2010; Analytical Luminescence Laboratory, San Diego, CA). Protein concentration was measured using a DC protein assay kit (Bio Rad Laboratories, Richmond, CA).

Results

Phenotype-specific effects of TNFa on human monocyte/macrophage apo E gene expression. Fig. 1 shows the effect of TNFa at 10 ng/ml on apo E gene expression in freshly isolated human monocytes. A four- to fivefold increase of apo E mRNA abundance resulted from treatment with TNFa over a 24-h period. Apo E gene expression in more fully differentiated macrophages (after 7 d in culture) showed little response to TNFa at 5 ng/ml.
Table I. Effect of TNFa on apo E mRNA Expression in Differentiated Macrophages

<table>
<thead>
<tr>
<th>Cell type</th>
<th>apo E/β-actin</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>THP1 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h control</td>
<td>2.58</td>
<td>1.0</td>
</tr>
<tr>
<td>24 h + TNFa</td>
<td>1.97</td>
<td>0.76</td>
</tr>
<tr>
<td>72 h control</td>
<td>2.46</td>
<td>1.0</td>
</tr>
<tr>
<td>72 h + TNFa</td>
<td>1.57</td>
<td>0.64</td>
</tr>
<tr>
<td>Human monocyte–derived</td>
<td></td>
<td></td>
</tr>
<tr>
<td>macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h control</td>
<td>3.88±0.14</td>
<td>1.0</td>
</tr>
<tr>
<td>48 h + TNFa</td>
<td>3.57±0.40</td>
<td>0.92</td>
</tr>
</tbody>
</table>

THP1 cells in 10% serum were treated with 100 ng/ml of PMA for 3 d. At that time the medium was replaced with 0.2% endotoxin-free BSA alone or with 5 ng/ml of TNFa for the indicated time. Human monocytes isolated by elutriation were allowed to differentiate in serum containing medium for 7 d. At that time, the medium was replaced with 0.2% endotoxin-free BSA alone or with 5 ng/ml TNFa for 48 h. Cells were analyzed by Northern blot by hybridization. The intensity of the bands for apo E and β-actin was measured by scanning densitometry. Results for THP1 cells are the average of duplicate samples, which varied <10%. Results from human monocyte–derived macrophage are mean±SD of triplicate samples.

Table II. Dose-dependent Effect of TNFa on apo E mRNA Expression

<table>
<thead>
<tr>
<th></th>
<th>apo E/β-actin</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.09</td>
<td>1</td>
</tr>
<tr>
<td>TNFa 5 ng/ml</td>
<td>0.32</td>
<td>3.6</td>
</tr>
<tr>
<td>TNFa 10 ng/ml</td>
<td>0.40</td>
<td>4.4</td>
</tr>
<tr>
<td>TNFa 15 ng/ml</td>
<td>0.42</td>
<td>4.7</td>
</tr>
</tbody>
</table>

20 μg total cellular RNA, isolated from untreated cells or cells treated with the indicated concentration of TNFα for 24 h, was analyzed by Northern hybridization. The intensity of the bands corresponding to apo E and β-actin was determined by scanning densitometry. The values shown are the averages of duplicate samples, which varied <10%.

Table III. Time Course Effect of TNFa on apo E mRNA Expression

<table>
<thead>
<tr>
<th>Time</th>
<th>apo E/β-actin</th>
<th>TNFa</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 h</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>24 h</td>
<td>0.07</td>
<td>0.16</td>
</tr>
<tr>
<td>72 h</td>
<td>0.09</td>
<td>1.26</td>
</tr>
</tbody>
</table>

20 μg of total cellular RNA isolated from untreated cells and cells treated with 5 ng/ml of TNFα for the indicated time was analyzed by Northern hybridization. The intensity of the bands corresponding to apo E and to the internal control, β-actin, was determined by scanning densitometry. The results shown are the averages of duplicate samples, which varied by <10%.

Figure 2. TNFα enhances apo E mRNA expression in early differentiating macrophages. THP1 cells in 0.2% endotoxin-free BSA were treated with 100 ng/ml of PMA alone or with 5 ng/ml of TNFα for 18 h. Northern blots of poly (A) + RNA for apo E and β-actin were processed as described in Methods. The bars show the change of apo E mRNA abundance after correcting for the β-actin signal.

TNFα Modulates apo E Gene Expression
with $[^{35}S]$methionine and quantitatively immunoprecipitating newly synthesized apo E from cell lysates and media. The results of a representative experiment are shown in Table IV. In culture media, apo E radioactivity was increased more than fourfold in response to a 72-h incubation in TNF-α. In cell lysates, there was a 1.8-fold increase in apo E radioactivity in response to TNF-α.

**Table IV. Effect of TNF-α on apo E Synthesis and Secretion**

<table>
<thead>
<tr>
<th></th>
<th>Medium</th>
<th>Cell extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>265±73</td>
<td>447±69</td>
</tr>
<tr>
<td>TNFα</td>
<td>1163±49</td>
<td>822±178</td>
</tr>
</tbody>
</table>

5 x 10⁵ THP1 cells in 60-mm dishes were incubated for 72 h in medium alone or with 5 ng/ml of TNF-α. The cells were then labeled for 2 h with $[^{35}S]$methionine to measure the rate of apo E synthesis and secretion as described in Methods. The apo E bands were excised from the gels, and radioactivity in apo E is shown in the lower panel as SEM from triplicate samples.

**Table V. Time Course Effect of TNF-α on c-fos and ICAM-1 mRNA Expression**

<table>
<thead>
<tr>
<th></th>
<th>c-fos/β-actin</th>
<th>β-actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>TNF-α 15 min</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>TNF-α 30 min</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>TNF-α 60 min</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>ICAM-1/β-actin</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>TNF-α 6 h</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>TNF-α 18 h</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>TNF-α 24 h</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>

20 µg of total cellular RNA was isolated from untreated cells or cells treated with 5 ng/ml of TNF-α for the indicated times and analyzed by Northern blot hybridization. The intensity of the bands for ICAM-1 or c-fos along with β-actin was determined by scanning densitometry. N.D. indicates that there was no c-fos or ICAM-1 signal detected for that experimental condition. Values shown are the average of duplicate samples, which varied by < 10%.

TNF-α effects can also be mediated by activation of pkC in transduction pathways separate from those involving ceramide (24–26). We therefore examined whether pathway inhibition of pkC activation during TNF-α treatment would attenuate apo E gene response. In the experiment shown in the bottom panel of Fig. 3, treatment with TNF-α produced a significant increase in apo E gene expression ($P < 0.02$). Concurrent treatment with the pkC inhibitor H7, 1-(5-isouquinolinesulfonyl)-2-methylpiperazine, completely prevented this induction however. A similar inhibition of TNF-α stimulation was produced by using staurosporine to inhibit pkC activity (not shown).

**Effect of TNF-α on apo E gene transcription.** We next considered the mechanism by which TNF-α increases apo E mRNA abundance. For other macrophage genes, TNF-α has been shown to increase mRNA abundance by stabilization of mRNA species without any change in gene transcription rate. To assess the contribution of increased apo E gene transcription, THP1 cells were transiently transfected with a chimeric construct containing apo E promoter sequences (from −2300 to +24 bp) fused to a luciferase reporter. After transfection, a single pool of cells was created to normalize transfection efficiency, and this pool was then aliquoted for incubations in TNF-α treatment for 24 and 48 h. TNF-α induced the expression of this chimeric construct 2.2-fold at 24 h and 2.1-fold at 48 h (Fig. 4).

**Discussion**

Our studies demonstrate that treatment with TNF-α stimulates apo E gene transcription, mRNA abundance, and apo E synthesis and secretion in monocyte/macrophage cells in a phenotype-dependent manner. Such concordant response at all major regulatory loci is not demonstrated by all macrophage targets of TNF-α modulation. For example, for FCγ Iib, TNF-α has been shown to decrease protein expression but increase mRNA abun-
dance in macrophages, and this increased mRNA abundance is due to a change in mRNA stability (21). While our studies establish that increased apo E gene transcription contributes to enhanced apo E mRNA abundance after TNFα treatment, a potential role for apo E mRNA stabilization is not excluded, especially for the large increase of apo E mRNA levels measured after prolonged incubation in TNFα (up to a 13-fold increase after 72 h). Alternatively, inclusion of additional apo E
gene elements may be necessary to maximize the transcriptional response of chimeric constructs to TNFα.

Others have studied the effect of TNFα on apo E expression with inconsistent results. Menju et al. investigated the effect of TNFα on THP1 cells after these cells were treated for 4 d with PMA, 12-0-tetradecarvolyphorbol-13-acetate (12). Additional treatment for 12 h in TNFα produced no effect on apo E mRNA abundance or protein synthesis. Zuckerman treated thioglycolate-elicited mouse peritoneal macrophages with TNFα for a longer period of time (48 h) and found an ~ 50% reduction of apo E protein secretion (10). Both of these reports are in accord with our observations regarding the phenotype-specific effect of TNFα on apo E gene expression. Monocyte/macrophages are multipotential cells that can respond to their humoral environment in divergent ways. Phenotype-dependent modulation of apo E expression by TNFα is consistent with the pleiotropic effects of TNFα that have been reported for other macrophage targets (22, 27). Such a phenotype-dependent response to cytokine stimulation may be related to activation of endogenous cytokine synthesis and secretion by the fully differentiated and activated macrophage. For example, PMA stimulation has been shown to increase TNFα mRNAs expression within 3 h and TNFα protein synthesis within 10 h in human monocytes (28). However, if endogenous synthesis of TNFα by differentiated macrophages accounts for the lack of apo E induction after treatment with exogenous TNFα, the suppression of apo E expression observed by Zuckerman et al. (10) and by us (Table 1) during prolonged incubations in TNFα would not be expected. Alternatively, phenotype-specific effects of TNFα may relate to differences in cell-matrix interactions between monocyte and macrophage phenotypes. Adhesion of cells to extracellular matrix has been shown to modify their response to TNFα (29). Further, in the vessel wall, it is likely that the presence of other cytokines will be involved in modulating monocyte/macrophage response to TNFα. Interferon-γ, for example, has been shown to be an important influence on the response of the IGF-1 expression after treatment of macrophages with TNFα (30).

TNFα treatment produced cell cycle arrest as indicated by a markedly reduced rate of DNA synthesis. In addition, TNFα increased the expression of c-fos within 15 min of its addition. Rapid and transient induction of c-fos expression serves as a marker for macrophage differentiation and/or activation (31). Also, TNFα enhanced the expression of the ICAM-1 gene. In arterial wall plaques, ICAM-1 expression is found in arterial smooth muscle cells and macrophages (32), and it has previously been shown that TNFα induces the expression of ICAM-1 in arterial smooth muscle cells (33). The effect of TNFα on apo E gene expression cannot be generalized to all cytokines associated with monocyte/macrophage activation or differentiation. Brand et al. added PMA and interferon-γ and observed no effect on apo E mRNA abundance in THP1 cells (34). THP1 cells have been shown to express MCSF receptors (35); however, in our laboratory, the addition of MCSF, with or without phorbol ester, to THP1 cells also had no effect on apo E mRNA abundance. This agrees with the report of Brand et al., who made a similar observation regarding the lack of MCSF effect on apo E protein synthesis and secretion in these cells (34).

Treating cells with TNFα has reportedly produced sphingomyelin turnover as well as endogenous cellular ceramide (24–26). Investigation of biologically important signaling transduction pathways for TNFα has focused on the role of ceramide as a second messenger as well as on a potential role for pκ activation. There are examples of pκ activation and ceramide producing opposing effects in target monocyte/macrophage cells (26). In HL60 cells, TNFα leads to increased activity of mitogen-activated protein kinase and the accumulation of nuclear factor–κB (NFκB) (36, 37). Both of these effects can be reproduced by exogenously added ceramide analogues. In Jurkat cells, TNFα decreases DNA synthesis and increases NFκB accumulation (38). In these cells, ceramide inhibits DNA synthesis but does not produce NFκB accumulation, although it potentiates the effect of TNFα on NFκB accumulation. These observations support a role for ceramide as a second messenger molecule mediating TNFα effects. Recently, however, the importance of ceramide as a TNFα second messenger has been
questioned. For example, Betts et al. directly measured ceramide levels, and they could find no difference produced by TNFα treatment in HL60 cells or Jurkat cells, even though NFκB activation was measurable in both cell types (39). Our data demonstrate that ceramide is not involved in TNFα modulation of the apo E gene. Addition of cell-permeant ceramide analogues did not increase apo E messenger RNA abundance nor did it potentiate the effect of TNFα on apo E. We also observed that treatment of THP1 cells with sphingomyelinase, to generate endogenous cellular ceramide, did not augment apo E mRNA abundance (not shown). Inhibitors of p38 activation, however, attenuated the effect of TNFα on apo E gene expression, suggesting the importance of this pathway for mediating TNFα effects on the apo E gene. With respect to the mechanism for TNFα induction of apo E gene transcription, it is noteworthy that TNFα treatment of THP1 cells induces expression of the c-fos gene. The protein product of this gene is a component of the AP1 transcription complex, which we have previously shown to be important for enhanced apo E gene transcription in the monocyte/macrophage (19).

In summary, our results document a significant role for TNFα in modulating monocyte/macrophage apo E gene expression. TNFα stimulates apo E gene transcription, mRNA abundance, and protein synthesis in the monocyte/macrophage in a phenotype-specific manner. This effect is observed in monocyte cells or cells in the early stage of monocyte/macrophage differentiation. In fully differentiated/activated macrophages, TNFα has no effect or reduces apo E mRNA abundance. Macrophages in vessel wall lesions are derived from the migration of bloodborne monocytes that cross the endothelium and transform, over time, into fully differentiated macrophages. Phenotypic monocytes as well as monocyte/macrophages in more advanced stages of differentiation have been identified in the vessel wall intima of hyperlipemic animals (40, 41). TNFα produced in the vessel wall could significantly modulate the abundance of apo E derived from monocytes/macrophages that are newly recruited into the vessel wall and thereby influence the genesis of the vessel wall lesion.

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