Induced expression of adipophilin mRNA in human macrophages stimulated with oxidized low-density lipoprotein and in atherosclerotic lesions

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Abstract Oxidized low-density lipoprotein (OxLDL) plays a critical role in foam cell formation and atherosclerogenesis. A cDNA encoding adipophilin was identified in cultured human macrophages stimulated with OxLDL using mRNA differential display. Adipophilin is a 50 kDa protein known to be a specific marker for adipocyte cell differentiation and lipid accumulation in a variety of cells. The time-dependent induction of adipophilin mRNA in macrophages was specific to OxLDL but not native LDL, and not to various cytokines and serum. In human atherosclerotic lesions, adipophilin mRNA expression was localized in a subset of lipid-rich macrophages. These data suggest that adipophilin-expressing macrophages may represent foam cells and this gene expression is likely to be associated with the lipid accumulation in foam cells of the atherosclerotic lesions.

Key words: Adipophilin; Oxidized low-density lipoprotein; Macrophage; Foam cell; Atherosclerosis

1. Introduction

Oxidized low-density lipoprotein (OxLDL) is known to play a key role in the induction of monocyte-derived macrophages into foam cells, and foam cell deposition in the vessel wall is one of the key characteristics in the development of human atherosclerotic lesions [1,2]. Uptake of OxLDL by the macrophages induces the expression of a wide range of growth factors and cytokines, many of which have been localized in atherosclerotic plaques [3–7]. A number of LDL binding proteins have also been found to be associated with foam cell formation and atherosclerosis, which include the macrophage scavenger receptor [8,9], LDL receptor-related protein [9], VLDL receptor [9] and CD36 [10]. However, the molecular mechanisms of their involvement in atherogenesis are not fully understood.

In an effort to identify other genes that are specifically induced in foam cell formation and to further understand the molecular mechanism of atherogenesis, we have applied an mRNA differential display method to identify genes that are induced in human macrophages in response to OxLDL stimulation. Using this approach, a cDNA previously known as adipose differentiation-related protein or adipophilin has been found. Adipophilin is a 50 kDa protein encoded by a gene initially isolated from differentiating adipocytes [11]. Initial tissue distribution of adipophilin mRNA expression in mice revealed its expression to be limited to adipose tissue [11]. Its expression has now been found in diverse cell types (such as fibroblasts, endothelial cells and epithelial cells) in culture and in particular, adipophilin is associated with the lipid fractions in the cell [12,13]. Adipophilin was also localized to specific cell types in tissues (such as lactating mammary epithelial cells, adrenal cortex cells, Sertoli and Leydig cells of the male reproductive system, and steatotic hepatocytes in alcoholic liver) [13]. These data suggest that adipophilin may be a specific marker for lipid accumulation in the cells.

A similar phenomenon of lipid accumulation occurs in human atherosclerotic lesion development. The early lipid core of an atherosclerotic lesion is associated with accumulation of lipid droplets rich in free cholesterol, while the later core development results in deposition of diverse types of lipid (including cholesterol, cholesterol esters and OxLDL) [14]. It has been shown that a large number of ruptured plaques were found to have contained a substantial lipid core, and macrophage foam cells dominated the site of rupture [15]. There is evidence to strongly suggest that death of macrophage foam cells may contribute to the origination and enlargement of the lipid core of an atherosclerotic lesion [16].

To elucidate the potential role of adipophilin in atherosclerosis, our present study compared its mRNA expression levels in cultured human macrophages stimulated with OxLDL to those stimulated with LDL, and further evaluated its expression in human atherosclerotic lesions using reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization techniques.

2. Materials and methods

2.1. Cell culture and treatment

Tissue culture medium for human macrophages was purchased from Gibco BRL. Penicillin, streptomycin, and human AB serum were from Sigma Chemical Co., St. Louis, MO, USA. Human recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) was obtained from Promega, Madison, WI, USA. Unmodified LDL and oxidized LDL were obtained from PerImmune, Inc., Rockville, MD, USA.

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Human monocytes were isolated from monocyte-enriched leukopacks (leukopheresis product, Biological Specialty Corp., Colmar, PA, USA) and purified by standard procedures as described [17]. Monocytes were plated at a density of approximately 2×10^5 cells/cm^2 into 150 cm^2 flasks, incubated in a CO_2 incubator at 37°C for 2 h to facilitate attachment, then washed to remove non-adherent lymphocytes. Monocytes were grown in RPMI 1640 medium containing 1 ng/ml GM-CSF, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 5% human AB serum to promote differentiation into macrophages. Cell culture medium was replaced twice weekly. Two week old cells in 150 cm^2 flasks were washed and incubated for 48 h in serum-free RPMI 1640 medium without GM-CSF, and then treated with 25 μg/ml of OxLDL or LDL for 1, 3, 6, 12, 24 and 48 h, 100 ng/ml lipopolysaccharide (LPS), 5 ng/ml tumor necrosis factor-α (TNF-α), 1 nM interleukin-1β (IL-1β) or 100 U/ml interferon-γ (IFN-γ) for 1, 3, 8 and 24 h, or 10% human AB serum for 0.5, 1, 3, 8 and 24 h. Medium was aspirated and cells were harvested with RNAzol B (Tel-Test, Inc., Friendswood, TX, USA), total RNA was prepared as per the manufacturer's suggestions, and was dissolved in DEPC-treated water.

2.2. mRNA differential display

Total cellular RNA isolated from macrophages stimulated with OxLDL for 0, 6 and 24 h was used for mRNA differential display. Reverse transcription reaction was carried out using an RNAmap kit (GenHunter, Nashville, TN, USA) with a specially designed 3′ primer matching the polyadenylation signal sequence (5′-NNNNNTTTATT-3′) [18]. Differential display PCR was carried out as described in detail previously [18,19]. The PCR products were resolved in an 8 M urea, 6% polyacrylamide DNA sequencing gel using a GenomyxLR DNA Sequencer (Foster City, CA, USA) and analyzed by autoradiography.

Differentially expressed DNA fragments were gel isolated, reamplified and subcloned into a pCRII vector. DNA sequencing was performed using a DNA Sequencer model 373A (Applied Biosystems) using universal primers of the cloning vector. DNA sequence was searched against GenBank database using a GCG program.

2.3. Northern hybridization analysis

RNA samples (30 μg/lane) were electrophoresed through formaldehyde-agarose slab gels and transferred to GeneScreen Plus membranes (DuPont-New England Nuclear). cDNA inserts of adipophilin and ribosomal protein L32 (rpL32) were released from plasmid DNA by restriction enzyme digestion and isolated after agarose gel electrophoresis. The DNA fragment was uniformly labeled with [α-^{32}P]dATP (3000 Ci/mmol, Amersham Corp.) using a random-priming DNA labeling kit (Boehringer Mannheim). Northern hybridization was carried out as described in detail previously [19]. The expression of the rpL32 gene is relatively constant in the present experimental condition and therefore was used to normalize the differences of the samples loaded in each lane.

2.4. Human tissue samples

Specimens for in situ hybridization were normal aorta ($n=3$) and renal artery ($n=2$) (obtained from the Anatomic Gift Foundation, White Oak, GA, USA), carotid endarterectomy specimens ($n=10$) from patients undergoing vascular reconstructive surgery for arterial occlusive disease and coronary arteries ($n=5$) from transplant recipients (from Department of Surgery, St. Thomas' Hospital, London, UK). Tissues were either mounted in OCT compound embedding medium (Agar Scientific Ltd, Essex, UK) and frozen in liquid nitrogen or snap-frozen in liquid nitrogen and mounted in OCT prior to cryostat sectioning. All specimens were stored at −70°C until further use.

2.5. RT-PCR

RNA was isolated from carotid endarterectomy specimens and reverse transcribed from 1 μg of DNase (Gibco BRL)-treated total RNA using Superscript II reverse transcriptase (Gibco BRL). For PCR, oligonucleotide primers for adipophilin and β-actin were designed in the coding region of the cDNA. The primers for adipophilin are 5′-AGGGGCTAGACAGGATTGAGGAGA-3′ (forward) and 5′-ACGGGAGTGAAGCTTGGTAGAC-3′ (reverse), representing bases 254–690 of the published sequence [20]. The primers for β-actin are 5′-ATCCCTGTACGCCTCTGG-3′ (forward) and 5′-TCCTTCTGCATCCTGTCG-3′ (reverse), representing bases 2085–2707. PCR was performed for 35 cycles (94°C for 1 min, 55°C for 1 min and 72°C for 30 s) and followed by one cycle of 72°C for 5 min. Negative controls were performed using human genomic DNA, water, and RT samples without reverse transcriptase as templates. Cloned plasmid DNA for adipophilin and β-actin was used as positive controls.

PCR products were analyzed by agarose gel electrophoresis. The gel was subsequently transferred onto a Hybond N$^+$ membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The blot was then hybridized with ^{32}P-labeled adipophilin probe in Church buffer (0.5 M Na phosphate buffer, 7% SDS, 0.5 mM EDTA) at 65°C for 16 h. After hybridization, the membrane was washed for 10 min in 2×SSC, 0.1% SDS and for 20 min in 0.2×SSC, 0.1% SDS at 65°C. Membranes were exposed to X-ray film for 15 min.

2.6. In situ hybridization

The adipophilin cDNA PCR product from the differential display was subcloned into pCRscript SK (Stratagene Ltd, Cambridge, UK). Sense and antisense RNA probes were labeled with [^{35}S]UTP (Amersham Pharmacia Biotech, Buckinghamshire, UK) using T7 or T3 polymerase (Promega, Southampton, UK), respectively. Prior to ethanol precipitation of the probe, 1 μl of the reaction was run on a 4% polyacrylamide/urea gel (Gibco BRL) to check for full length transcripts.

Transverse tissue sections (10 μm) were thaw-mounted onto Superfrost$^+$ microscope slides (BDH, Leicestershire, UK). Sections were fixed in fresh 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, acetylated in 0.25% acetic anhydride/0.1 M triethanolamine/0.1 M NaCl and dehydrated and delipidated through a graded series of alcohol and chloroform. Sections were air-dried and stored at −70°C until use.

Sense and antisense riboprobes were resuspended at 25 000 cpm/μl in hybridization buffer (50% formamide, 0.02% w/v bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 100 μg/ml polyadenylate, 100 μg/ml denatured salmon sperm DNA, 100 μg/ml yeast tRNA, 4×SSC, 10% dextran sulfate, 10 mM dithiothreitol) and slides were incubated overnight in a sealed humid chamber at 55°C. After hybridization, the sections were washed with 1×SSC at room temperature for 30 min, treated with 20 μg/ml RNase A in buffer and then with buffer alone (500 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 30 min each at 37°C), washed with 1×SSC (room temperature, 30 min), followed by a high stringency wash (0.5×SSC at 65°C for 30 min) and 0.5×SSC at room temperature for 2×10 min. Slides were dehydrated through a series of alcohol, air-dried and dipped in photographic emulsion (Amersham Pharmacia Biotech). Slides were exposed for 8 weeks at 4°C and developed using Kodak D19 (1:1 water), counterstained in toluidine blue, dehydrated through a graded series of alcohol and coverslipped for microscopic analysis.

2.7. Immunohistochemistry

The cellular composition of the plaques was determined using a panel of characterized antibodies: HHF35 (specific for smooth muscle α-actin), CD68, a specific macrophage marker, von Willebrand factor for endothelial cells (all from Dako Ltd., High Wycombe, Buckinghamshire, UK) and CD3 leu, a marker for T-cells (Becton Dickinson, Oxford, UK).

Serial transverse tissue sections (10 μm) were thaw-mounted onto Superfrost$^+$ microscope slides, re-frozen and stored at −70°C until use. For immunohistochemistry (IHC), sections were air-dried for 1 h and fixed in acetone for 10 min. IHC was performed using the avidin-biotin-peroxidase complex method. All incubations were done at room temperature unless otherwise stated. Briefly, endogenous peroxidase activity was blocked by incubating slides in 0.6% H_2O_2 in methanol. Non-specific binding was blocked by incubating sections in normal rabbit serum (Dako) before applying the primary antibody. Optimal primary antibody concentrations were pre-determined by titration and slides were incubated for 60 min in the appropriate primary antibody dilutions. Slides were then incubated in biotinylated rabbit anti-mouse secondary antibody (Dako) for 30 min, washed and incubated with avidin/biotinylated horseradish peroxidase complex for 30 min. Slides were then stained with diaminobenzidine tetrahydrochloride substrate. The sections were counterstained with hematoxylin, dehydrated through a graded series of alcohol and mounted in DPX. Negative controls were omission of primary antibody and substitution of primary antibody with appropriate isotype control.

2.8. Lipid staining

Frozen sections were air-dried for 1 h and then fixed in 4% paraformaldehyde/PBS for 10 min at room temperature. Slides were rinsed briefly in 60% isopropanol and then incubated for 15 min in fresh filtered oil red O solution (BDH, UK) (60% saturated oil red O/isopropanol/0.4% dextrin). Slides were then washed in 60% isopropanol, rinsed in tap water, counterstained with hematoxylin and mounted in glycerol/gelatin solution (Sigma, UK).

3. Results

3.1. Identification of adipophilin mRNA upregulation in human macrophages stimulated with OxLDL by mRNA differential display

Fig. 1A illustrates a representative autoradiograph of differential display PCR using cultured macrophages stimulated with OxLDL. A band designated ADP was strongly induced in macrophages stimulated with OxLDL as shown on the mRNA differential display sequencing gel (Fig. 1A). The same expression profile of ADP was confirmed by Northern analysis (Fig. 1B).

DNA sequencing analysis and database searching of this particular clone revealed that it shares over 99% nucleotide sequence identity with a human cDNA sequence (corresponding to the bases of 288–796 of X97324 [20]) that encodes adipocyte differentiation-related protein or adipophilin. One-base mismatch was found in both 5′ primer (C instead of T at the most 5′-end) and 3′-anchored degenerated primers (A instead of G at the fifth base of the 3′-end) to the published sequence [20].

3.2. Time-dependent induction of adipophilin mRNA in OxLDL- but not LDL-induced human macrophages

Fig. 2 illustrates the adipophilin mRNA expression in human macrophages stimulated with OxLDL and LDL. A relatively low but constitutive level of adipophilin mRNA expression was observed in unstimulated macrophages. Its expression was gradually increased upon OxLDL stimulation and reached a highest level after 48 h (approximately four-fold increase compared to the basal level, as quantitated using PhosphorImage analysis of the Northern blots). In contrast, unmodified LDL only induced a very low, inconsistent level of adipophilin mRNA expression in human macrophages (Fig. 2).

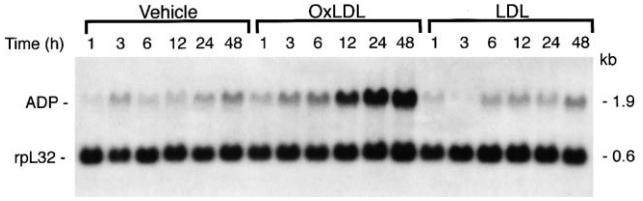

Fig. 2. Time-course expression of adipophilin mRNA in cultured human macrophages stimulated with OxLDL and LDL. Cultured human macrophages were challenged with OxLDL or LDL for 0, 3, 6, 12, 24 and 48 h as described in Section 2. Total cellular RNA was isolated and resolved (30 μg/lane) by electrophoresis, transferred to a nylon membrane, and hybridized to adipophilin (ADP) and rpL32 cDNA probe as indicated. The autoradiogram was exposed for 14 h. The same experiments were repeated three times and similar results were obtained.

3.3. Expression of adipophilin mRNA in human macrophages stimulated with cytokines and serum

Since cytokines and possibly growth factors are known to play an important role in foam cell formation and atherogenesis, the effect of these mediators on adipophilin mRNA expression in cultured human macrophages was examined. A representative study is illustrated in Fig. 3. A constitutive basal expression of adipophilin mRNA was observed in macrophages; TNF-α, IL-1β, IFN-γ, LPS or serum failed to induce its expression.

3.4. Detection of adipophilin mRNA in atherosclerotic plaques by RT-PCR

To determine whether adipophilin mRNA was expressed in

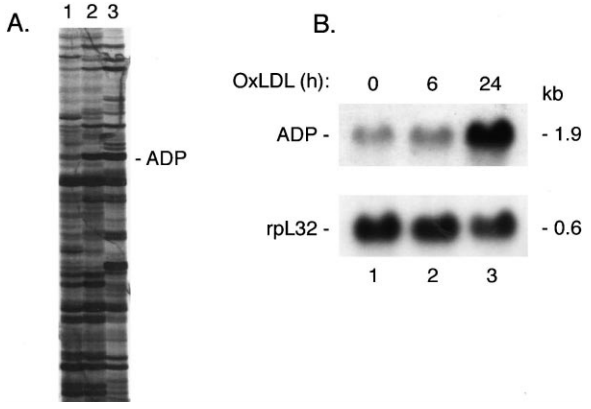

Fig. 1. Identification of upregulated adipophilin in cultured human macrophages stimulated with OxLDL using mRNA differential display. Upstream primer (5′-CCTGAATCAG-3′) in combination with downstream primers (5′-NNNNNNTAAAT-3′) was used for differential display to examine the gene expression in human macrophages stimulated with OxLDL for 0 (lane 1), 6 (lane 2) and 24 h (lane 3). The band indicated with an arrow (ADP) was isolated, reamplified and used for Northern analysis (B). The housekeeping gene, ribosomal protein L32 (rpL32), was used to show the differences of total cellular RNA loaded in each lane.

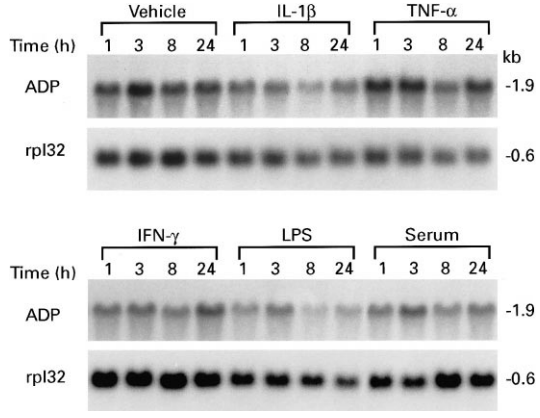

Fig. 3. Expression of adipophilin mRNA in cultured human macrophages stimulated with IL-1β, TNF-α, IFN-γ, LPS and serum. Cultured human macrophages were serum-deprived and challenged with IL-1β, TNF-α, IFN-γ, LPS or serum for 1, 3, 8 and 24 h as described in Section 2. Total cellular RNA (30 μg/lane) was resolved by electrophoresis and Northern hybridized to adipophilin (ADP) and rpL32 cDNA probes. The autoradiograms for adipophilin hybridization were exposed for 48 h to enhance the signal. Similar experiments were repeated three times and adipophilin mRNA expression was not induced by any of these factors.

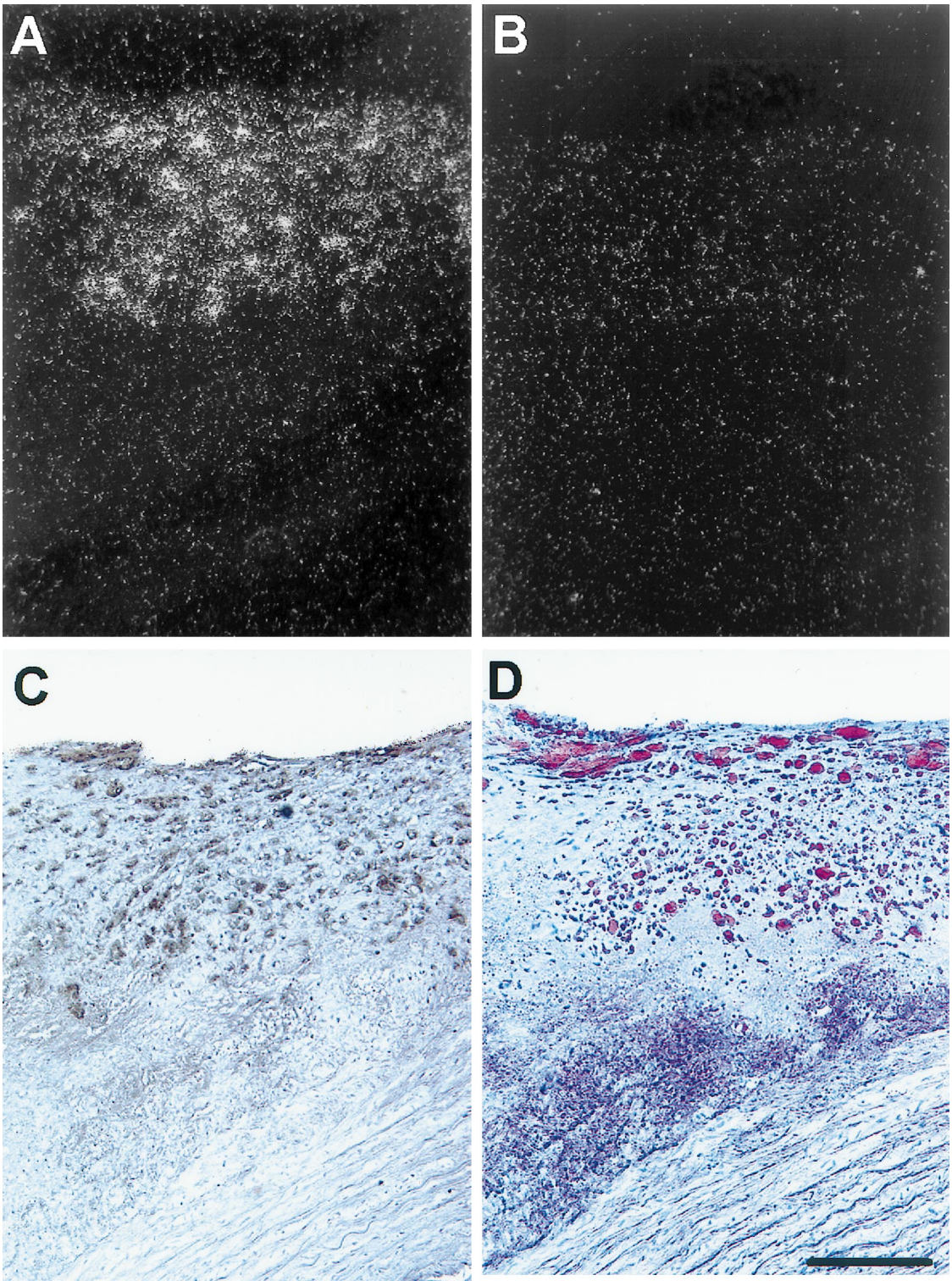

Fig. 4. Serial sections of a carotid endarterectomy specimen show the adipophilin mRNA expression in a macrophage/lipid-rich area of the specimen. A: Dark-field photomicrograph showing in situ hybridization for adipophilin mRNA using the specific antisense probe. B: In situ hybridization of adipophilin sense riboprobe. C: Immunohistochemical staining for macrophages using anti-CD68 antibodies. D: Oil red O staining showing the lipid deposition in the tissue. Bar represents 200 μm.

atherosclerotic arteries, we screened a number of cDNAs derived from carotid endarterectomies ($n = 6$) using gene-specific primers for adipophilin. To test for specificity, the PCR gel was blotted onto a nylon membrane and probed with ^{32}P-labelled adipophilin cDNA. β-Actin was used to assess the quality of the RNA. A specific adipophilin PCR product was observed from all of the atherosclerotic specimens examined (data not shown).

3.5. In situ localization of adipophilin mRNA in human macrophages of the atherosclerotic plaques

Since adipophilin mRNA expression was upregulated in macrophages during foam cell formation and its expression was detected in carotid endarterectomies by PCR, we further examined its expression in both normal and diseased arteries using in situ hybridization. A strong signal for adipophilin mRNA was observed in a number of carotid endarterectomy specimens (six out of 10); its expression was localized mainly in a highly occluded coronary artery with a macrophage-rich core (see Fig. 4 for the representative carotid endarterectomy specimen). No signal above background was observed in the three normal aorta specimens or in four out of five coronary arteries that displayed only slight intimal thickening. To determine the cellular composition of the lesions and determine the cell types expressing adipophilin mRNA, immunohistochemical staining was carried out in serial sections with markers for SMC (α-actin), macrophages (CD68), endothelial cells (von Willebrand factor) and T-cells (CD3) (results not shown). Adipophilin mRNA was expressed in and limited to macrophage-rich areas of the lesions (compare Fig. 4A with C). Staining of serial sections with oil red O revealed these regions to be rich in cell-associated lipid (Fig. 4D). Medial smooth muscle cells or adventitial macrophages of normal vessels may express low levels of adipophilin mRNA although it could not be detected using our current method.

4. Discussion

In the present study we identified the induced expression of adipophilin mRNA in human macrophages stimulated with OxLDL. This induction was specific for OxLDL but not LDL, suggesting a potential role of this gene product in foam cell formation. In addition, the localization of elevated adipophilin mRNA expression in human atherosclerotic lesions, and in particular in a subset of macrophages that may represent foam cells in the lesions, suggests a role of this gene product in the pathogenesis of atherosclerosis.

Other genes have been identified to be upregulated in foam cell formation and associated with atherosclerosis, including various cytokines, growth factors, tissue remodeling proteins, and the receptors for LDL/OxLDL [3–9]. To test the possibility that these upregulated cytokines and growth factors may contribute to the adipophilin gene expression, we evaluated adipophilin mRNA expression in cultured human macrophages stimulated with inflammatory mediators (LPS, IL-1β, TNF-α and IFN-γ) and serum. None of these factors appears to be the inducer of adipophilin mRNA expression in the cultured macrophages (Fig. 3).

OxLDL was recently demonstrated to activate macrophage gene expression through a member of the nuclear hormone receptor family, peroxisome proliferator activator receptor γ (PPARγ) [21]. PPARs are known to regulate fat cell development, lipid and glucose metabolism, and they are implicated in metabolic disorders, such as hypertriglyceridemia and diabetes, which can lead to atherosclerosis. PPARγ is also expressed in macrophage-derived foam cells of human atherosclerotic lesions [22] and PPARγ ligands such as fatty acids are components of OxLDL [23]. PPARγ has been shown to drive adipocyte differentiation [24] and promote OxLDL uptake through transcriptional induction of scavenger receptor CD36 [25], a gene highly expressed in foam cells within human atherosclerotic aorta [26]. Therefore, it is likely that the OxLDL-induced upregulation of adipophilin that we have observed may be a consequence of the stimulation of PPARγ.

It should be pointed out that the role of adipophilin in foam cells and in atherosclerosis is only suggestive, especially the levels of the transcript to be translated into protein in macrophages remain to be demonstrated. Previous findings of its appearance in cells with triacylglycerol-rich lipid accumulation suggest that adipophilin may play a key role in foam cell lipid metabolism and contribute to the development of the lipid-rich core in human atherosclerosis. One can speculate that as OxLDL induces its own uptake mechanism, it may concomitantly induce adipophilin to handle lipid storage. Adipophilin may either represent an actively contributing manifestation of atherosclerosis, or be a protective attempt to mobilize lipid away from the developing lesion. It is possible that adipophilin may occupy a key modulatory role to balance lipid sequestration with mobilization to regulate the susceptibility of vulnerable plaques. Study of the regulation of adipophilin function in macrophages may provide a therapeutic opportunity for the treatment of atherosclerosis.

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References

[1] Ross, R. (1986) New Engl. J. Med. 314, 488–500.
[2] Quinn, M.T., Parthasarathy, S., Fong, L.G. and Steinberg, D. (1997) Proc. Natl. Acad. Sci. USA 84, 2995–2998.
[3] Clinton, S.K. and Libby, P. (1992) Arch. Pathol. Lab. Med. 116, 1292–1300.
[4] Ross, R. (1993) Nature 362, 801–809.
[5] Nilsson, J. (1993) Cardiovasc. Res. 27, 1184–1190.
[6] Wang, N., Tabas, I., Winchester, R., Ravalli, S., Rabbani, L.E. and Tall, A. (1996) J. Biol. Chem. 271, 8837–8842.
[7] Ramos, M.A., Kuzuya, M., Esaki, T., Miura, S., Satake, S., Asai, T., Kanda, S., Hayashi, T. and Iguchi, A. (1998) Arterioscler. Thromb. Vasc. Biol. 18, 1188–1196.
[8] Han, J.H. and Nicholson, A.C. (1998) Am. J. Pathol. 152, 1647–1654.
[9] Hiltunen, T.P., Luoma, J.S., Nikkari, T. and Ylaherttuala, S. (1998) Circulation 97, 1079–1086.
[10] Han, J.H., Hajjar, D.P., Febbraio, M. and Nicholson, A.C. (1997) J. Biol. Chem. 272, 21654–21659.
[11] Jiang, H.P. and Serrero, G. (1992) Proc. Natl. Acad. Sci. USA 89, 7856–7860.
[12] Brasaemle, D.L., Barber, T., Wolins, N.E., Serrero, G., Blanchettemackie, E.J. and Londos, C. (1997) J. Lipid Res. 38, 2249–2263.
[13] Heid, H.W., Moll, R., Schwetlick, I., Rackwitz, H.R. and Keenan, T.W. (1998) Cell Tissue Res. 294, 309–321.
[14] Guyton, J.R. and Klemp, K.F. (1996) Arterioscler. Thromb. Vasc. Biol. 16, 4–11.
[15] van der Wal, A.C., Becker, A.E., van der Loos, C.M. and Das, P.K. (1994) Circulation 89, 36–44.
[16] Ball, R.Y., Stowers, E.C., Burton, J.H., Cary, N.R.B., Skepper, J.N. and Mitchinson, M.J. (1995) Atherosclerosis 114, 45–54.
[17] Lysko, P.G., Weinstock, J., Webb, C.L., Brawner, M.E. and Elshourbagy, N.A. (1999) J. Pharmacol. Exp. Ther. 289, 1277–1285.
[18] Wang, X.K., Li, X. and Feuerstein, G.Z. (1998) BioTechniques 24, 382–386.
[19] Wang, X.K., Yue, T.L., Ohlstein, E.H., Sung, C.P. and Feuerstein, G.Z. (1996) J. Biol. Chem. 271, 24286–24293.
[20] Heid, H.W., Schnolzer, M. and Keenan, T.W. (1996) Biochem. J. 320, 1025–1030.

[21] Nagy, L., Tontonoz, P., Alvarez, J.G.A., Chen, H.W. and Evans, R.M. (1998) Cell 93, 229–240.
[22] Ricote, M., Huang, J., Fajas, L., Li, A., Welch, J., Najib, J., Witztum, J.L., Auwerx, J., Palinski, W. and Glass, C.K. (1998) Proc. Natl. Acad. Sci. USA 95, 7614–7619.
[23] Kliewer, S.A., Sundseth, S.S., Jones, S.A., Brown, P.J., Wisely, G.B., Koble, C.S., Devchand, P., Wahli, W., Willson, T.M., Lenhard, J.M. and Lehmann, J.M. (1997) Proc. Natl. Acad. Sci. USA 94, 4318–4323.
[24] Saladin, R., Fajas, L., Dana, S., Halvorsen, Y.D., Auwerx, J. and Briggs, M. (1999) Cell Growth Differ. 10, 43–48.
[25] Tontonoz, P., Nagy, L., Alvarez, J.G.A., Thomazy, V.A. and Evans, R.M. (1998) Cell 93, 241–252.
[26] Nakata, A., Nakagawa, Y., Nishida, M., Nozaki, S., Miyagawa, J., Nakagawa, T., Tamura, R., Matsumoto, K., Kameda-Takemura, K., Yamashita, S. and Matsuzawa, Y. (1999) Arterioscler. Thromb. Vasc. Biol. 19, 1333–1339.