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Low Density Lipoprotein Receptor-related Protein Mediates Apolipoprotein E Inhibition of Smooth Muscle Cell Migration*

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This research was undertaken to identify the cell surface receptor responsible for mediating apolipoprotein E (apoE) inhibition of platelet-derived growth factor (PDGF)-directed smooth muscle cell migration. Initial studies revealed the expression of the low density lipoprotein receptor (LDLR), the LDL receptor-related protein (LRP), the very low density lipoprotein receptor (VLDL), and apoE receptor-2 in mouse aortic smooth muscle cells. Smooth muscle cells isolated from LDLR-null, VLDL-null, and apoE receptor-2-null mice were responsive to apoE inhibition of PDGF-directed smooth muscle cell migration, suggesting that these receptors were not involved. An antisense RNA expression knockdown strategy, utilizing morpholino antisense RNA against LRP, was used to reduce LRP expression in smooth muscle cells to assess the role of this receptor in apoE inhibition of cell migration. Results showed that apoE was unable to inhibit PDGF-directed migration of LRP-deficient smooth muscle cells. The role of LRP in mediating apoE inhibition of PDGF-directed smooth muscle cell migration was confirmed by experiments showing that antibodies against LRP effectively suppressed apoE inhibition of PDGF-directed smooth muscle cell migration. Taken together, these results document that apoE binding to LRP is required for its inhibition of PDGF-directed smooth muscle cell migration.

The importance of apolipoprotein E (apoE)[1] in vascular protection has been recognized for a number of years (1, 2). Aside from its well documented function in mediating lipoprotein clearance by the liver (3), recent evidence suggests that apoE also has direct cell regulatory functions in manners that are protective against vascular disease (4, 5). These include the modulation of inflammatory response by suppressing lymphocyte activation (6, 7), inhibiting agonist-induced platelet aggregation and activation (8, 9), and suppressing growth factor-induced smooth muscle cell migration and proliferation (10, 11). ApoE is also present in the brain and the central nervous system, where it participates in neurite outgrowth and nerve regeneration (12, 13). These diverse functions of apoE may possibly be attributed to its ability to bind several different receptors on cell surface membranes.

The initial step of apoE-mediated cholesterol transport is its binding to the LDL receptor on the cell surface (14). Subsequent steps include endocytosis of the apoE-containing lipoproteins and their transport to lysosomes where lipoprotein degradation takes place. Unesterified cholesterol liberated from lysosomal degradation of lipoproteins can then be used for maintenance of cellular cholesterol homeostasis (14). The LDL receptor is located in clathrin-coated pits, and its rapid endocytosis is mediated through the NP*X*Y motif on the cytoplasmic tail of the protein (15). In the absence of a functional LDL receptor, apoE interaction with the LDL receptor-related protein (LRP) may also deliver cholesterol to cells through a similar endocytosis/lysosomal processing mechanism (16–20). In contrast to the LDL receptor, the two NP*X*Y motifs within the cytoplasmic tail of the LRP play only a minimal role in endocytosis (21). The dominant endocytosis signal in LRP is conferred by a Y*XX*L motif and a dileucine sequence (21).

The initial step of apoE modulation of cell functions is also mediated by its binding to the cell surface. Previous studies have shown that apoE and immunoregulatory LDL suppress lymphocyte activation via binding to cell surface receptors, but endocytosis is not a prerequisite (6, 22, 23). Although the immunosuppressive apoE receptor has not been identified to date, it is quite clear that the LDL receptor is not involved (24). ApoE stimulation of neurite outgrowth is also independent of the LDL receptor and is mediated by apoE interaction with LRP (13, 25). Importantly, both immunosuppression and stimulation of neurite outgrowth by apoE are unrelated to its cholesterol-transporting properties because minimally lipidated or delipidated apoE are equally active in regulating these lymphocyte and neuronal cell functions (6, 22, 26). In contrast, apoE inhibition of platelet aggregation requires its presence in a lipoprotein form (9). Nevertheless, the LDL receptor does not appear to play a role in apoE inhibition of platelet aggregation (8). ApoE inhibition of platelet aggregation is also unrelated to its cholesterol transport function (8) but has been suggested to be mediated through binding to another member of the LDL receptor gene family, the apoE receptor 2 (apoER2), on the platelet membrane (8, 27) and through the induction of nitric oxide synthesis (9).

The mechanism by which apoE inhibits smooth muscle cell migration and proliferation is also attributed to its ability to modulate cell signaling events (10, 11). Previously, we showed that apoE inhibits growth factor-induced smooth muscle cell

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[1] The abbreviations used are: apoE, apolipoprotein E; LDL, low density lipoprotein; LRP, low density lipoprotein receptor-related protein; PDGF, platelet-derived growth factor; DMEM, Dulbecco's modified Eagles' medium; MAP kinase, mitogen-activated protein kinase; uPA, urokinase-type plasminogen activator; VLDLR, very low density lipoprotein receptor.

migration and proliferation via inhibition of mitogen-activated protein kinase and induction of cyclin D1 expression (10). Moreover, apoE inhibition of smooth muscle cell proliferation, but not their migration, appears to be mediated by stimulation of nitric-oxide synthase (11). The latter observation suggested that apoE inhibits smooth muscle cell migration and proliferation through different mechanisms. Our recent data confirmed this hypothesis and revealed that apoE inhibition of smooth muscle cell proliferation is mediated through its binding to heparan sulfate proteoglycans, whereas its inhibition of cell migration is mediated through binding to cell surface receptors. Although the heparan sulfate proteoglycan perlecan has been implicated as a mediator for apoE inhibition of cell proliferation (28), the identity of the cell surface receptor responsible for apoE inhibition of smooth muscle cell migration remains elusive. The goal of this study is to identify the receptor involved.

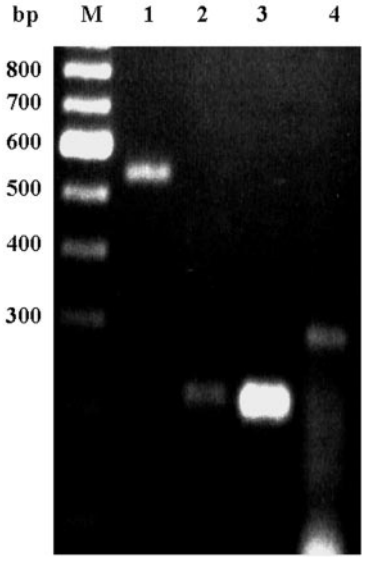


FIG. 1. **Reverse transcriptase-polymerase chain termination reaction analysis of smooth muscle cell mRNA.** Total cellular RNA was isolated from mouse aortic smooth muscle cells and used as templates for reverse transcriptase-polymerase chain termination reaction with oligonucleotide primers for mouse LRP (*lane 1*), VLDL receptor (*lane 2*), LDL receptor (*lane 3*), and apoE receptor-2 (*lane 4*). The reaction products were analyzed by electrophoresis on 1.5% agarose gels. The migration of the product was compared with standard 100-base pair DNA size markers (*M*). The markers on the gel range from 800–300 base pairs as indicated.

EXPERIMENTAL PROCEDURES

Materials—Type I collagenase and elastase were obtained from Sigma. Dulbecco's modified Eagles' medium (DMEM), fetal bovine serum, PDGF-BB, SuperScript II reverse transcriptase, and *Taq* polymerase were purchased from Invitrogen. The RNA extraction reagent RNA Stat-60 was obtained from Tel-Test "B," Inc. (Friendswood, TX). Transwell polycarbonate membrane filters were obtained from Corning Costar Corp. (Cambridge, MA). Antibodies against LRP were generated against purified human placental LRP as described (29). The anti-LRP IgG was characterized previously and shown to inhibit apoE binding to LRP on mammalian cells (13, 25). The hybridoma cell line IgG-11H4 that secretes mouse monoclonal antibodies against the carboxyl terminus of LRP (30) was obtained from ATCC (Manassas, VA). These hybridoma cells were originally produced in the Brown and Goldstein laboratory and were characterized previously (30). Morpholino-derivatized oligonucleotides were purchased from Gene Tools (Corvallis, OR).

Isolation of Mouse Aortic Smooth Muscle Cells—Aortic smooth muscle cells were isolated from either C57BL/6 wild type mice or mice that are defective in LDL receptor, VLDL receptor, or apoE receptor-2 gene expression. All the animals were obtained from Jackson Laboratories (Bar Harbor, ME), except the apoE receptor-2 knockout mice, which were the gift of Dr. Joachim Herz at the University of Texas Southwestern Medical School. Aortic smooth muscle cells were isolated using a modification of the procedure used by Mimura *et al.* (31) and characterized as described previously (32). The primary aortic smooth muscle cells were cultured in DMEM containing 10% fetal bovine serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Cells between passages 1 and 5 were used for experiments.

Reverse Transcription and Polymerase Chain Reaction of Receptor mRNA—Total RNA was isolated from primary smooth muscle cells by the guanidine thiocyanate/phenol/chloroform extraction method (33) with the RNA-Stat 60 reagent. 2 μg of total RNA was reverse-transcribed into cDNA by SuperScript II reverse transcriptase and amplified by polymerase chain reaction. The polymerase chain reaction primers were designed based on published sequences of mouse LDL receptor, LRP, VLDL receptor, and apoE receptor 2, respectively (34–37), as follows: LDL receptor (bases 208–413), 5′ primer, 5′-TCCAATCAATTCAGCTGTGG-3′, and 3′ primer, 5′-GAGCCATCTAGGCAATCTCG-3′; LRP (bases 275–811), 5′ primer, 5′-CGGAACTGTACCATTTCA-3′, and 3′ primer, 5′-GGTGTTGACAACCCATTCG-3′; VLDL receptor (bases 286–487), 5′ primer, 5′-CTCCCAGTTTCAGTGCACAA-3′ and 3′ primer, 5′-ATCAGAACCGTCTTCGCAAT-3′; and apoE receptor 2 (bases 325–604), 5′ primer, 5′-GGAGATGCGATGAGGACAAC-3′, and 3′ primer, 5′-CCACCCTCACAGTCCTTCTC-3′. The reaction was conducted with an initial denaturation step at 94 °C for 4 min, followed by 35 cycles, each consisting of incubation at 94 °C for 30 s, 58 °C for 30 s, and 74 °C for 45 s. The size of the PCR product was assessed by electrophoresis on 1.5% agarose gels.

Smooth Muscle Cell Transfection—The morpholino antisense oligonucleotides were transfected into primary smooth muscle cells using

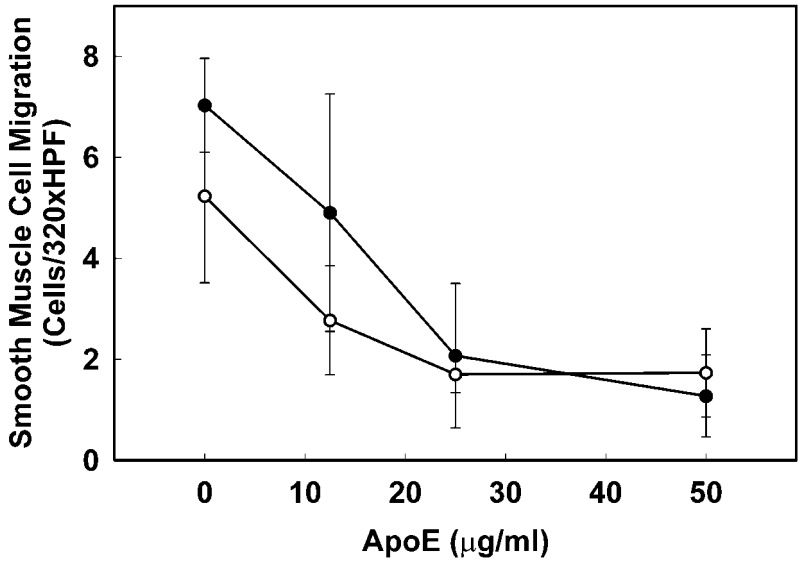


FIG. 2. **Effect of apoE on PDGF-directed smooth muscle cell migration.** Quiescent mouse smooth muscle cells isolated from aortas of normal wild type mice (*filled circles*) or mice with LDL receptor gene deletion (*open circles*) were incubated with apoE for 30 min at 37 °C prior to adding the cells to the top chamber of Transwell membranes at a density of 6×10^4 cells/well in a 24-well dish. The lower chamber of the plate contained basal medium and 10 ng/ml PDGF-BB. Cells were incubated in the Transwells for 4 h at 37 °C. The number of cells that migrated to the lower surface was counted in different high power fields. Data are expressed as mean ± S.D. (n = 6).

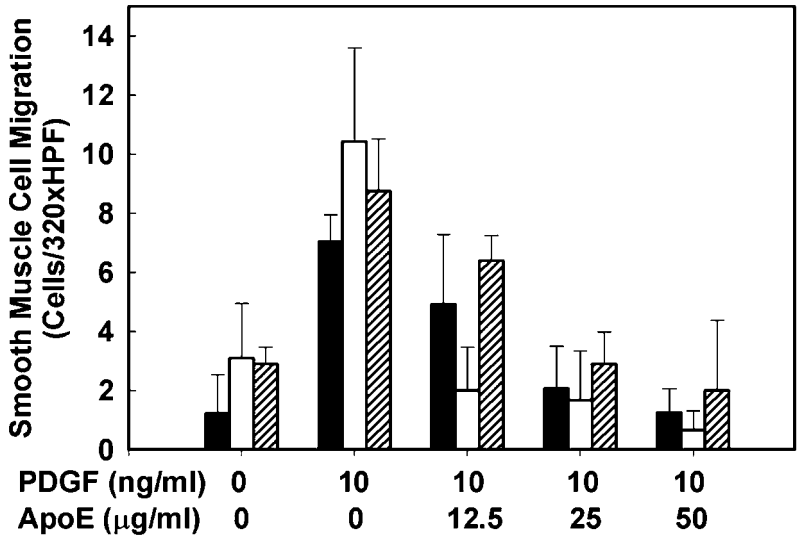


FIG. 3. **Effect of apoE on PDGF-directed smooth muscle cell migration.** Mouse smooth muscle cells were isolated from aortas of normal wild type mice (*solid bars*) or mice with defective VLDL receptor (*open bars*) or apoE receptor-2 (*hatched bars*) genes. The cells were made quiescent by incubation for 24 h in media containing 0.4% fetal bovine serum. The quiescent cells were then incubated with apoE for 30 min at 37 °C prior to adding the cells to the top chamber of Transwell membranes at a density of 6×10^4 cells/well in a 24-well dish. The lower chamber of the plate contained basal medium and 10 ng/ml PDGF-BB. Cells were incubated in the Transwells for 4 h at 37 °C. The number of cells that migrated to the lower surface was counted in different high power fields. Data are expressed as mean ± S.D. ($n = 6$).

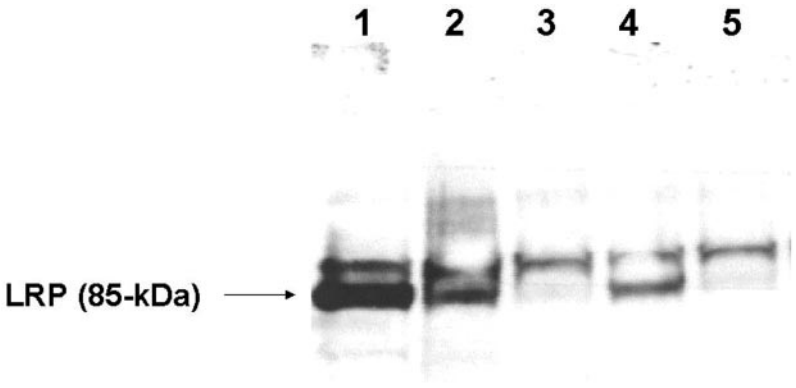


FIG. 4. **Antisense RNA knockdown of LRP expression in mouse smooth muscle cells.** Mouse smooth muscle cells cultured in 60-mm dishes were incubated for 3 h with 3 ml of DMEM containing either 14 (condition A) or 17 (condition B) μl of a 500 μM solution of morpholino oligonucleotide and 14 (condition A) or 17 (condition B) μl of the EPEI transfection reagent from Gene Tools. Cells were washed and then incubated overnight with DMEM containing 10% fetal bovine serum prior to inducing quiescence by 24 h incubation in DMEM containing 0.4% fetal bovine serum. Proteins were extracted from quiescent transfected cells and electrophoresed in 10% SDS-polyacrylamide gels. *Lane 1* contained proteins from untransfected smooth muscle cells; *lanes 2* and *3* contained proteins from cells transfected with either random or antisense LRP morpholino oligonucleotides under condition A, respectively, and *lanes 4* and *5* contained proteins from cells transfected with either random or antisense LRP morpholino oligonucleotides under condition B, respectively. The samples were transferred to nitrocellulose membranes and immunoblotted with the 11H4 monoclonal antibody that recognizes the 85-kDa subunit of LRP. The band above the 85-kDa LRP band is a protein that crossreacts with the secondary antibody.

the special delivery agent and instructions as provided by Gene Tools. The cells were made quiescent 24 h after oligonucleotide transfection by incubation for 48 h with DMEM and 0.4% fetal bovine serum. For each experiment, half of the transfected quiescent smooth muscle cells were used for migration studies as described below. The remaining cells were centrifuged at $500 \times g$ for 10 min, resuspended in SDS-polyacrylamide gel electrophoresis loading buffer, and stored at −80 °C until analyzed by Western blot analysis with monoclonal antibody 11H4 against the 85-kDa subunit of LRP.

Smooth Muscle Cell Migration—Smooth muscle cell migration toward PDGF-BB was evaluated according to the procedure of Law *et al.* (38), as described previously (32). Briefly, 6×10^4 quiescent smooth muscle cells in 0.3 ml of DMEM were incubated with or without apoE and in the presence or absence of antibodies for 30 min at 37 °C. An aliquot (0.1 ml) of the incubation mixture was then added to the top chamber of tissue culture-treated Transwell polycarbonate membrane with 8-μM pores in 24-well plates. The lower Transwell compartment contained 0.6 ml of DMEM, 0.4% fetal bovine serum, and 0.2% bovine serum albumin, with or without 10 ng/ml PDGF-BB. After incubating the sample for 4 h at 37 °C, the upper surface of the Transwell was washed with phosphate-buffered saline. Cells attached to the membranes were fixed with methanol for 10 min at 4 °C followed by hematoxylin staining. The number of cells that migrated to the lower surface of the filter was determined by counting the cells in six high power fields (×320). Maximum cell migration was determined as the number of cells that migrated toward PDGF at the bottom of the Transwell chamber when incubation was carried out in the absence of apoE. All experiments were performed in triplicate and were repeated at least three times.

Apolipoprotein E Isolation—Human apoE was isolated from pooled fresh plasma obtained from healthy volunteers by the method of Cardin *et al.* (39). The purity of apoE was assessed by SDS-polyacrylamide gel electrophoresis. Samples containing a single band with $M_r = 34{,}000$ were used. The purified apoE was stored as a lyophilized sample at −80 °C and was resuspended in phosphate-buffered saline immediately prior to use. The apoE was not reconstituted with lipids prior to its addition to cells.

RESULTS

Previous reports show the presence of the LDL receptor, LRP, VLDL receptor, and apoE receptor-2 on human, rabbit, and rat smooth muscle cells (40–42). The presence of these LDL receptor family members on mouse aortic smooth muscle cells was verified by reverse transcriptase-PCR amplification of their RNA (Fig. 1). Because a common characteristic of LDL receptor family members is the ability to bind apoE, one or more of these receptors may mediate apoE inhibition of smooth muscle cell migration in response to growth factor stimulation.

The role of the LDL receptor in apoE inhibition of PDGF-stimulated smooth muscle cell migration was assessed by de-

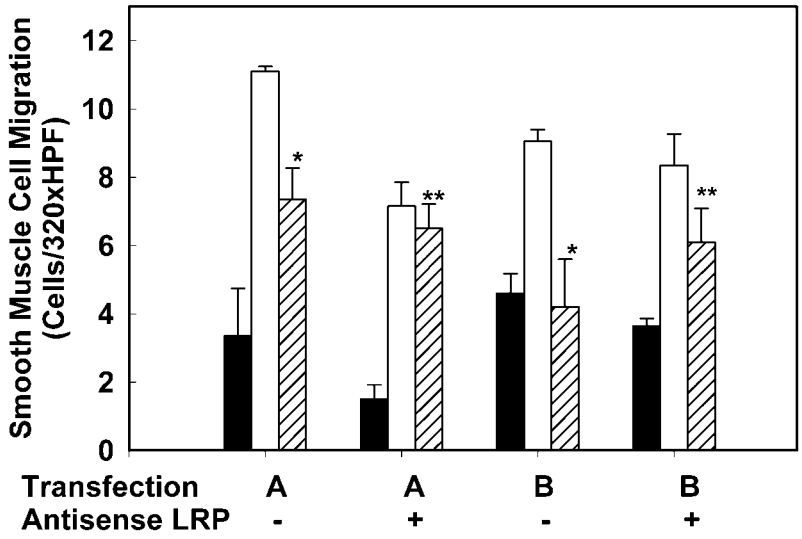
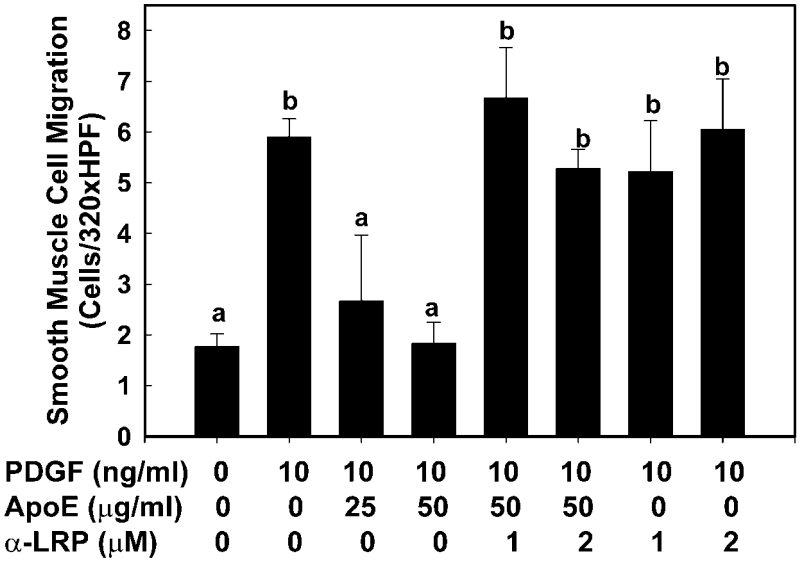


FIG. 5. **Effect of apoE on PDGF-stimulated migration of LRP-deficient smooth muscle cells.** Mouse aortic smooth muscle cells were transfected with random or antisense LRP morpholino oligonucleotides as described in the legend to Fig. 4. Quiescent transfected cells were then incubated with or without 50 μg/ml apoE and placed in the top chamber of the Transwell in which the bottom chamber contained 10 ng/ml PDGF-BB. Cell migration was determined after 4 h. The *solid bars* show results of migration of cells transfected under Conditions A or B with either morpholino antisense LRP oligonucleotides (+) or morpholino random sequence nucleotides (−) incubated in the absence of apoE and without PDGF stimulation. The *open bars* show results of PDGF-directed migration of cells transfected with either random (−) or antisense LRP (+) under conditions A or B in the absence of apoE pretreatment. The *hatched bars* show results of PDGF-directed migration of cells transfected with either random (−) or antisense LRP (+) under conditions A or B after preincubation with apoE. The data represent mean ± S.D. from triplicate determinations in 2 separate experiments. * indicates difference from PDGF-stimulated migration at $p < 0.05$. ** indicates no significant difference from PDGF-stimulated migration.

FIG. 6. **Effect of anti-LRP on PDGF-stimulated migration of mouse aortic smooth muscle cells.** Serum-starved wild type mouse smooth muscle cells were preincubated in the presence or absence of apoE and anti-LRP for 30 min at 37 °C prior to the addition to the top chamber of Transwell membranes in 24-well dishes at a density of 6×10^4 cells/well. Cells were allowed to migrate toward the bottom chamber containing PDGF-BB at the concentrations indicated. Cells that migrated to the lower surface of the membranes were counted in different high power fields. Data represent the mean ± S.D. from three triplicate determinations in two separate experiments. *Bars* with different letters are significantly different from each other at $p < 0.05$.



termining apoE inhibition of PDGF-directed migration of smooth muscle cells isolated from $LDLR^{+/+}$ and $LDLR^{-/-}$ mice. The results showed that whereas smooth muscle cells lacking the LDL receptors were less efficient in migration toward PDGF compared with cells containing the LDL receptor, apoE effectively inhibited PDGF-directed migration of smooth muscle cells obtained from both $LDLR^{+/+}$ and $LDLR^{-/-}$ mice (Fig. 2). These results documented that apoE inhibition of smooth muscle cell migration is not because of its binding to the LDL receptor.

A similar approach was used to assess the role of the VLDL receptor and apoE receptor-2 on apoE inhibition of smooth muscle cell migration. Smooth muscle cells isolated from $VLDLR^{-/-}$ mice and from apoE receptor-2-negative mice were similar to cells from wild type mice in their response to apoE inhibition of smooth muscle cell migration (Fig. 3). Thus, neither the VLDL receptor nor the apoE receptor-2 was responsible for this apoE effect.

The role of LRP in mediating apoE inhibition of smooth muscle cell response to PDGF cannot be addressed using a similar approach because of the lethality of the $LRP^{-/-}$ mice. To circumvent this problem, an antisense receptor knockdown approach was used to produce LRP-deficient smooth muscle cells. An antisense oligonucleotide 5′-CCTCTGGTCCTGTTACTTCTTGTCC-3′, which is complementary to LRP mRNA residues 386–410, was synthesized for these experiments. The oligonucleotide was modified by morpholino linkage to increase solubility and to provide highly specific antisense activity in transfected cells (43). The incubation of primary smooth muscle cells with this morpholino-derivatized antisense LRP oligonucleotide resulted in cells that were defective in expression of LRP (Fig. 4). As a control, cells incubated with a random

sequence oligonucleotide had no effect on LRP expression (Fig. 4). The random morpholino oligonucleotide also had no effect on the subsequent ability of the cells to migrate toward PDGF (Fig. 5). In contrast, preincubating the smooth muscle cells with the morpholino-derivatized antisense LRP oligonucleotide significantly abrogated the apoE effect on suppression of PDGF-directed smooth muscle cell migration (Fig. 5). The ability of the antisense LRP oligonucleotide to suppress apoE inhibition of cell migration in each experiment correlated well with its effect on LRP expression (Fig. 5). These data strongly suggest that LRP is the cell surface receptor that mediates apoE inhibition of smooth muscle cell migration.

Additional experiments using a different approach were used to confirm the role of LRP in mediating apoE inhibition of cell migration. Polyclonal antibodies that were shown previously to inhibit apoE binding to LRP (13, 25) were used in these experiments. The results of these studies showed that anti-LRP antibodies alone have no direct effect on PDGF-stimulated smooth muscle cell migration (Fig. 6). However, the anti-LRP antibodies significantly alleviated apoE inhibition of smooth muscle cell migration toward PDGF (Fig. 6).

DISCUSSION

Results of the current study documented that apoE inhibition of PDGF-directed smooth muscle cell migration is mediated via its binding to LRP. This conclusion is based on the observations that (i) apoE failed to inhibit PDGF-directed migration of cells with suppressed LRP expression because of antisense knockdown, and (ii) the antimigratory property of apoE was abolished by antibodies against LRP. The LRP-mediated apoE suppression of smooth muscle cell migration is specific to this member of the LDL receptor family proteins because apoE was capable of suppressing migration of cells lacking LDL receptor, VLDL receptor, or apoE receptor-2.

The demonstration of a functional role of LRP in modulating smooth muscle cell migration is consistent with results reported previously by other investigators. Okada *et al.* (44) showed that inhibition of LRP function, either by anti-LRP or by the receptor-associated protein RAP, reduced smooth muscle cell migration in response to the urokinase-type plasminogen activator uPA. Although uPA is capable of binding to LRP on smooth muscle cells (45), its promotion of cell migration appeared to be mediated through a distinct uPA receptor (44). The binding of uPA to LRP was proposed to serve only as a clearance mechanism for protease and protease/inhibitor complexes (46, 47). Okada *et al.* (44) have suggested that LRP may promote uPA-induced smooth muscle cell migration by clearing uPA and allowing free uPA receptor to return to the cell surface to participate in the stimulatory process. However, there is no direct demonstration that LRP inhibition will decrease uPA receptor expression on the cell surface. In fact, fibroblasts with genetic defects in LRP expression have increased uPA receptor activity and accelerated migration on vitronectin (48). Fibrosarcoma cells lacking LRP also displayed increased levels of the uPA receptor with a concomitant increase in migration on vitronectin-coated surfaces (49). Thus, it is possible that anti-LRP and receptor-associated protein (RAP) inhibition of uPA-induced smooth muscle cell migration observed in the earlier studies by Okada *et al*. (44) was a direct effect of the LRP receptor. Our results demonstrating that apoE binding to LRP inhibited PDGF-directed smooth muscle cell migration are consistent with this interpretation. However, it must be noted that anti-LRP alone had no effect on PDGF-stimulated cell migration in our study, whereas anti-LRP was reported to inhibit uPA-stimulated cell migration (44). The difference between our results and those reported earlier is unclear but may be related to differences between uPA and PDGF in the mechanism of stimulation of cell migration or to the reactivity differences between the two anti-LRP antibodies used in the respective experiments.

It is interesting to note that LRP binding to either apoE (current study) or RAP (44) resulted in inhibition of agonist-induced smooth muscle cell migration. These results suggest that occupancy of the extracellular domain of LRP is sufficient to confer resistance to agonist-induced smooth muscle cell migration. Whereas the mechanism by which ligand binding to LRP results in inhibition of smooth muscle cell migration remains unclear at this time, the data add to an expanding literature that suggests LRP may mediate signal transduction events in addition to its role as an endocytosis receptor protein (50, 51). Recently, yeast two-hybrid assays revealed LRP interaction with an extensive set of cytoplasmic adaptor or scaffold proteins in the brain (52). In addition, LRP has also been shown to be tyrosine phosphorylated, possibly at one or both of its NP*X*Y domains, and LRP phosphorylatioin results in its interaction with the cytoplasmic adaptor protein Shc (53). Interestingly, most of these LRP-interacting proteins contain PTB or PDZ domains with suggested functions in the regulation of MAP kinases, cell adhesion, vesicle trafficking, or neurotransmission (52). We have previously shown that apoE inhibits PDGF-induced MAP kinase activity in smooth muscle cells (10). In view of the requirement of MAP kinase activation for PDGF-stimulated smooth muscle cell migration (54), it is possible that apoE binding to LRP may promote LRP phosphorylation, resulting in the recruitment of MAP kinase and disruption of the PDGF receptor signaling cascade. Additional experiments are necessary to test this hypothesis.

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REFERENCES

1. Mahley, R. W. (1988) *Science* **240,** 622–630
2. Curtiss, L. K., and Boisvert, W. A. (2000) *Curr. Opin. Lipidol.* **11,** 243–251
3. Mahley, R. W., and Ji, Z. S. (1999) *J. Lipid Res.* **40,** 1–16
4. Curtiss, L. K. (2000) *Arterioscler. Thromb. Vasc. Biol.* **20,** 1852–1853
5. Swertfeger, D. K., and Hui, D. Y. (2001) *Frontiers in Bioscience* **6,** d526.-d535
6. Hui, D. Y., Harmony, J. A. K., Innerarity, T. L., and Mahley, R. W. (1980) *J. Biol. Chem.* **255,** 11775–11781
7. Pepe, M. G., and Curtiss, L. K. (1986) *J. Immunol.* **136,** 3716–3723
8. Riddell, D. R., and Owen, J. S. (1996) *Thromb. Res.* **81,** 597–606
9. Riddell, D. R., Graham, A., and Owen, J. S. (1997) *J. Biol. Chem.* **272,** 89–95
10. Ishigami, M., Swertfeger, D. K., Granholm, N., and Hui, D. Y. (1998) *J. Biol. Chem.* **273,** 20156–20161
11. Ishigami, M., Swertfeger, D. K., Hui, M. S., Granholm, N. A., and Hui, D. Y. (2000) *Arterioscler. Thromb. Vasc. Biol.* **20,** 1020–1026
12. Nathan, B. P., Bellosta, S., Sanan, D. A., Weisgraber, K. H., Mahley, R. W., and Pitas, R. E. (1994) *Science* **264,** 850–852
13. Fagan, A. M., Bu, G., Sun, Y., Daugherty, A., and Hotzman, D. M. (1996) *J. Biol. Chem.* **271,** 30121–30125
14. Brown, M. S., and Goldstein, J. L. (1983) *J. Clin. Invest.* **72,** 743–747
15. Chen, W. J., Goldstein, J. L., and Brown, M. S. (1990) *J. Biol. Chem.* **265,** 3116–3123
16. Lund, H., Takahashi, K., Hamilton, R. L., and Havel, R. J. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 9318–9322
17. Hussain, M. M., Maxfield, F. R., Mas-Oliva, J., Tabas, I., Ji, Z. S., Innerarity, T. L., and Mahley, R. W. (1991) *J. Biol. Chem.* **266,** 13936–13940
18. Choi, S. Y., and Cooper, A. D. (1993) *J. Biol. Chem.* **268,** 15804–15811
19. Willnow, T. E., Sheng, Z., Ishibashi, S., and Herz, J. (1994) *Science* **264,** 1471–1474
20. Yu, K. C. W., Chen, W., and Cooper, A. D. (2001) *J. Clin. Invest.* **107,** 1387–1394
21. Li, Y., Marzolo, M. P., van Kerkhof, P., Strous, G. J., and Bu, G. (2000) *J. Biol. Chem.* **275,** 17187–17194
22. Hui, D. Y., and Harmony, J. A. K. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 4764–4768
23. Harmony, J. A. K., and Hui, D. Y. (1981) *Cancer Res.* **41,** 3799–3802
24. Curtiss, L. K., and Edgington, T. S. (1978) *J. Clin. Invest.* **61,** 1298–1308
25. Holtzman, D. M., Pitas, R. E., Kilbridge, J., Nathan, B., Mahley, R. W., Bu, G., and Schwartz, A. L. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9480–9484
26. DeMattos, R. B., Curtiss, L. K., and Williams, D. L. (1998) *J. Biol. Chem.* **273,** 4206–4212
27. Riddell, D. R., Vinogradov, D. V., Stannard, A. K., Chadwick, N., and Owen, J. S. (1999) *J. Lipid Res.* **40,** 1925–1930
28. Paka, L., Goldberg, I. J., Obunike, J. C., Choi, S. Y., Saxena, U., Goldberg, I. D.,

and Pillarisetti, S. (1999) *J. Biol. Chem.* **274,** 36403–36408
29. Bu, G., Maksymovitch, E. A., and Schwartz, A. L. (1993) *J. Biol. Chem.* **268,** 13002–13009
30. Kowal, R. C., Herz, J., Goldstein, J. L., Esser, V., and Brown, M. S. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 5810–5814
31. Mimura, Y., Kobayashi, S., Notoya, K., Okabe, M., Kimura, I., Horikoshi, I., and Kimura, M. (1995) *Biol. Pharm. Bull.* **18,** 1373–1376
32. Swertfeger, D. K., and Hui, D. Y. (2001) *J. Biol. Chem.* **276,** 25043–25048
33. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159
34. Hoffer, M. J., van Eck, M. M., Petrij, F., van der Zee, A., de Wit, E., Meijer, D., Grosveld, G., Havekes, L. M., Hofker, M. H., and Frants, R. R. (1993) *Biochem. Biophys. Res. Commun.* **191,** 880–886
35. Van Leuven, F., Stas, L., Raymakers, L., Overbergh, L., De Strooper, B., Hilliker, C., Lorent, K., Fias, E., Umans, L., and Torreskens, S. (1993) *Biochim. Biophys. Acta* **1173,** 71–74
36. Gafvels, M. E., Paavola, L. G., Boyd, C. O., Nolan, P. M., Wittmaack, F., Chawla, A., Lazar, M. A., Bucan, M., Angelin, B. O., and Strauss, J. F. (1994) *Endocrinology* **135,** 387–394
37. Kim, H. J., Kim, D. H., Magoori, K., Saeki, S., and Yamamoto, T. T. (1998) *J. Biochem.* **124,** 451–456
38. Law, R. E., Meehan, W. P., Xi, X. P., Graf, K., Wuthrich, D. A., Coats, W., Faxon, D., and Hsueh, W. A. (1996) *J. Clin. Invest.* **98,** 1897–1905
39. Cardin, A. D., Holdsworth, G., and Jackson, R. L. (1984) in *Methods in Pharmacology* (Schwartz, A., ed) Vol. 5, pp. 141–166, Plenum Publishing Co., New York
40. Yla-Herttuala, S. (1996) *Curr. Opin. Lipidol.* **7,** 292–297
41. Yamamoto, T., and Bujo, H. (1996) *Curr. Opinion Lipid.* **7,** 298–302
42. Schneider, W. J., Nimpf, J., and Bujo, H. (1997) *Curr. Opin. Lipidol.* **8,** 315–319
43. Summerton, J., and Weller, D. (1997) *Antisense Nucleic Acid Drug Develop.* **7,** 187–195
44. Okada, S. S., Grobmyer, S. R., and Barnathan, E. S. (1996) *Arterioscler. Thromb. Vasc. Biol.* **16,** 1269–1276
45. Grobmyer, S. R., Kuo, A., Orishimo, M., Okada, S., Cines, D. B., and Barnathan, E. S. (1993) *J. Biol. Chem.* **268,** 13291–13300
46. Orth, K., Madison, E. L., Gething, M. J., Sambrook, J. F., and Herz, J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 7422–7426
47. Nykjaer, A., Petersen, C. M., Moller, B., Jensen, P. H., Moestrup, S. K., Holtet, T. L., Etzerodt, M., Thogersen, H. C., Munch, M., Andreasen, P. A., and Gliemann, J. (1992) *J. Biol. Chem.* **267,** 14543–14546
48. Weaver, A. M., Hussaini, I. M., Mazar, A., Henkin, J., and Gonias, S. L. (1997) *J. Biol. Chem.* **272,** 14372–14379
49. Webb, D. J., Nguyen, D. H. D., and Gonias, S. L. (2000) *J. Cell Sci.* **113,** 123–134
50. Gliemann, J. (1998) *Biol. Chem.* **379,** 951–964
51. Herz, J. (2001) *Neuron* **29,** 571–581
52. Gotthardt, M., Trommsdorff, M., Nevitt, M. F., Shelton, J., Richardson, J. A., Stockinger, W., Nimpf, J., and Herz, J. (2000) *J. Biol. Chem.* **275,** 25616–25624
53. Barnes, H., Larsen, B., Tyers, M., and van der Geer, P. *(2001) J. Biol. Chem.* **276,** 19119–19125
54. Cospedal, R., Abedi, H., and Zachary, I. (1999) *Cardiovasc. Res.* **41,** 708–721