THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 270, No. 37, Issue of September 15, pp. 21672–21678, 1995
Printed in U.S.A.

Synergistic Effects of Growth Factors on the Regulation of Smooth Muscle Cell Scavenger Receptor Activity*

(Received for publication, May 26, 1995, and in revised form, July 3, 1995)

Qingqing Gong and Robert E. Pitas‡§

From the Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, and the ‡Department of Pathology, University of California, San Francisco, California 94141-9100

Rabbit smooth muscle cells (SMC) express types I and II scavenger receptors (ScR) that are up-regulated by platelet secretion products. In the current studies we investigated the effect of growth factors secreted by platelets on ScR activity in rabbit and human SMC. Platelet-derived growth factor (PDGF BB) and transforming growth factor β_1 (TGF-β_1) at 10 ng/ml increased ScR activity in rabbit SMC (by approximately 4- and 2-fold, respectively) but not in human SMC. Epidermal growth factor (EGF) or insulin-like growth factor I (IGF-I) alone had little effect on SMC ScR activity. The growth factors had synergistic effects on ScR activity and on types I and II ScR mRNA expression. In rabbit SMC, PDGF BB, EGF, and TGF-β_1 together stimulated ScR activity 12-fold. In human SMC, EGF and TGF-β_1, together with either IGF-I or PDGF BB, stimulated receptor activity approximately 7-fold. Growth factor-mediated induction of ScR activity in rabbit and human SMC was blocked by the tyrosine kinase inhibitor tyrphostin 47, whereas the induction of ScR activity in rabbit but not human SMC was blocked by the protein kinase C inhibitor MDL.29,152. Studies using neutralizing antibodies demonstrated that TGF-β_1 is the predominant factor in *in vitro* preparations of platelet secretory products which regulates ScR activity. The growth factors that act synergistically in regulating ScR activity *in vitro* are all present in atherosclerotic lesions, where they are produced by macrophages, endothelial cells, SMC, and platelets. The data suggest that these growth factors may regulate ScR activity in SMC *in vivo* and contribute to foam cell formation.

One characteristic feature of atherosclerotic lesions is the unregulated accumulation of lipoprotein-derived cholesterol and cholesteryl esters in macrophages and smooth muscle cells of the arterial intima. Lipid is deposited in these cells as droplets that give the cells a foamy appearance when viewed by phase-contrast microscopy (1–4). The mechanism of lipid accumulation and foam cell formation is not known with certainty; however, lipid accumulation in macrophages has been postulated to result from the scavenger receptor-mediated internalization of modified lipoproteins (5–8). We have demonstrated recently that smooth muscle cells also express scavenger receptors, and we have postulated a similar mechanism for lipid accumulation in these cells (9–12).

The expression of the scavenger receptor in both macrophages and smooth muscle cells can be induced during atherogenesis. Whereas circulating monocytes do not express scavenger receptors, scavenger receptor expression is induced to high levels when the monocytes adhere to the endothelium, penetrate between the endothelial cells, and differentiate to macrophages in the subendothelial space (2, 4, 13, 14). During atherogenesis, smooth muscle cells migrate from the media to the intima of the arterial wall, where they proliferate and accumulate lipid, becoming foam cells. Scavenger receptor activity is detected in smooth muscle cells in the intima but not in normal vascular smooth muscle cells (15–17), demonstrating that smooth muscle cell scavenger receptor activity is up-regulated in atherosclerotic lesions. The factors contributing to the regulation of smooth muscle cell scavenger receptor activity *in vivo* are unknown.

We have shown that scavenger receptor activity in rabbit smooth muscle cells is induced *in vitro* by incubation of the cells with phorbol esters, serum, or secretion products from platelets (10). The expression of types I and II scavenger receptors in smooth muscle cells is normally quite low; however, incubation of the cells with phorbol esters increases receptor activity up to 20-fold (10–12). Types I and II scavenger receptor cDNAs have been cloned from a cDNA library prepared from phorbol ester-treated rabbit smooth muscle cells (18). Incubation of rabbit smooth muscle cells with secretion products from human platelets, stimulated with thrombin or with the calcium ionophore A23187, also results in up to a 5–6-fold increase in receptor activity (10–12). These data suggest that platelet secretory products present in serum or at sites of platelet aggregation may contribute to the regulation of smooth muscle cell scavenger receptor activity.

In the present studies we examined the effect of several known platelet secretory products on scavenger receptor activity in human and rabbit smooth muscle cells. The results demonstrate that platelet-derived growth factor (PDGF)[1] BB, epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), and transforming growth factor β_1 (TGF-β_1), growth factors present in atherosclerotic lesions (19–23), synergistically stimulate types I and II scavenger receptor gene expression and receptor activity in both human and rabbit smooth muscle cells.

* This work was supported in part by National Institutes of Health Program Project Grant HL47660. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Gladstone Institute of Cardiovascular Disease, P. O. Box 419100, San Francisco, CA 94141-9100. Tel.: 415-826-7500; Fax: 415-285-5632.

[1] The abbreviations used are: PDGF, platelet-derived growth factor; EGF, epidermal growth factor; IGF-I, insulin-like growth factor I; TGF-β_1, transforming growth factor β_1; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; LDL, low density lipoproteins; Ac-LDL, acetyl low density lipoproteins; FACS, fluorescence-activated cell sorter; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ.

EXPERIMENTAL PROCEDURES

Materials—Heat-inactivated fetal bovine serum was obtained from HyClone Laboratories (Logan, UT). Dulbecco's modified Eagle's medium, Dulbecco's phosphate-buffered saline, penicillin, and streptomycin were obtained from Life Technologies, Inc. Two human aortic smooth muscle cell lines and growth medium with (SmGM 2) or without (SmBM) serum were purchased from Clonetics (San Diego). Serotonin, fibrinogen, and fibronectin were purchased from Sigma. Human recombinant EGF, IGF-I, PDGF, and TGF-β_1 were purchased from Life Technologies, Inc. Polyclonal anti-PDGF antibody and monoclonal antibodies against EGF and IGF-I were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). Polyclonal antibody against TGF-β_1 was obtained from Promega (Madison, WI). Tyrphostin 47 and tyrphostin 1 were purchased from Boehringer Mannheim and BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA), respectively. The protein kinase C inhibitor MDL.29,152 (4-propyl-5-(4-quinolinyl)-2-[^{3}H]-oxazolone) was a gift from the Marion Merrell Dow Research Institute (Cincinnati, OH) (24). Fresh human platelet-rich plasma was obtained from the Peninsula Blood Bank (San Mateo, CA). The fluorescent probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Molecular Probes, Inc. (Eugene, OR). Sodium [^{125}I]iodide was purchased from Amersham Corp.

Lipoproteins—Low density lipoproteins (LDL) (d = 1.02–1.05 g/ml) were obtained from human plasma (1 mg/ml EDTA) by sequential density gradient ultracentrifugation and then dialyzed against saline EDTA (0.15 M NaCl, 0.01% EDTA) (25). The LDL were iodinated by the method of Bilheimer *et al.* (26) or labeled with the fluorescent probe DiI as described (27, 28). The ^{125}I-LDL and DiI-labeled LDL were then acetylated as described (29).

Cell Culture Experiments—The New Zealand White rabbit smooth muscle cell line SMC.3 was provided by Drs. Lisa Minor and George Rothblat at the Medical College of Pennsylvania in Philadelphia. Human smooth muscle cells were grown in SmGM 2. Rabbit smooth muscle cells were grown in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). For experiments, the medium was aspirated, and the cells were preincubated at 37 °C for 16 h in serum-free Dulbecco's modified Eagle's medium or SmBM containing either growth factors or platelet secretory products prepared as described (10). The cells were then washed three times with SmBM or Dulbecco's modified Eagle's medium, incubated with DiI-labeled acetyl-LDL (Ac-LDL) (5 μg/ml) for 8 h at 37 °C, and processed for fluorescence-activated cell sorter (FACS) analysis as described (10).

For lipoprotein metabolism assays, the cells were treated with growth factors as described above. After three washes with serum-free medium, the cells were incubated with ^{125}I-labeled Ac-LDL (5 μg/ml) at 37 °C for 16 h. Cell-associated radioactivity (*i.e.* bound and internalized lipoprotein) and trichloroacetic acid-soluble lipoprotein degradation products in the medium were quantitated as described (30). Nonspecific degradation (*i.e.* the amount of degradation obtained in the presence of a 100-fold excess of unlabeled Ac-LDL) has been subtracted from all data.

RNase Protection Assay—A specific DNA template was generated by polymerase chain reaction amplification from a vector containing rabbit type II scavenger receptor cDNA sequences (18) (a gift from Dr. Mason Freeman of Massachusetts General Hospital, Boston). Amplification was accomplished using oligonucleotide primers corresponding to bases 764–783 (sense) and 1014–1041 (antisense) of the rabbit scavenger receptor cDNA. A 29-base T7 promoter sequence was added at the 5′ end of the antisense primer, and a random 21-base sequence was added to the 5′ end of the sense primer. A ^{32}P-labeled antisense RNA probe was generated using T7 polymerase. The labeled probe of the expected size (307 bases) was purified by gel electrophoresis. The RNase protection assays were performed on total RNA isolated from cells. Using this probe, a 277-base pair fragment will be protected by mRNAs for both the types I and II scavenger receptor.

Western Blot Analysis and Neutralization Experiments—For Western blot analysis, platelet secretory products (40–60 μg) were separated on a nonreducing sodium dodecyl sulfate-polyacrylamide (15%) slab gel and electrophoretically transferred to nitrocellulose (12, 31). The nitrocellulose membranes were then subjected to immunoblotting using antibodies against PDGF, EGF, IGF-I, and TGF-β_1 according to the manufacturer's instructions. Recombinant PDGF BB, EGF, IGF-I, and TGF-β_1 were used as positive controls and in the quantitation of growth factors in the platelet secretory products.

To neutralize their activity, platelet secretory products (40 μg/ml) were preincubated for 1 h at 37 °C with blocking antibodies to the growth factors (concentrations indicated in the legend to Fig. 4) before addition to the cells. The concentrations of anti-PDGF and anti-TGF-β_1 antibodies used were sufficient to block the effect of recombinant PDGF BB (50 ng/ml) and TGF-β_1 (20 ng/ml) on scavenger receptor activity in rabbit smooth muscle cells, respectively. The concentrations of anti-IGF-I and anti-EGF antibodies used were sufficient to block the effect of IGF-I (20 ng/ml) and EGF (10 ng/ml) on smooth muscle cell proliferation measured by assaying the mitochondrial dehydrogenase activity as described (Boehringer Mannheim).

RESULTS

Our previous studies have shown that types I and II scavenger receptors are expressed by rabbit smooth muscle cells and that incubation of the cells with platelet secretory products up-regulates receptor activity (10). Upon activation, platelets have been reported to secrete a number of products, including fibronectin, fibrinogen, serotonin, and the growth factors PDGF, EGF, TGF-β_1, and IGF-I (32). To determine whether these secretory products stimulate scavenger receptor activity in smooth muscle cells, we preincubated rabbit or human smooth muscle cells with them for 16 h at 37 °C and examined the uptake of DiI-labeled Ac-LDL by fluorescence microscopy. Fibrinogen (10–70 ng/ml), fibronectin (10–70 ng/ml), and serotonin (10–70 ng/ml) had little effect on scavenger receptor activity, whereas preincubation with the combination of the four growth factors significantly enhanced the uptake of DiI-labeled Ac-LDL by both rabbit and human smooth muscle cells (data not shown).

To obtain a quantitative estimate of the increase in receptor activity induced by the growth factors and to assess the effect of each growth factor individually, additional experiments were performed. The cells were preincubated with the recombinant growth factors either alone or in combination, and the uptake of DiI-labeled Ac-LDL was assessed by FACS analysis. When DiI-labeled lipoproteins are internalized by cells and degraded, the DiI is quantitatively retained in the lysosomes. The amount of DiI in the cells is therefore directly proportional to the amount of lipoprotein metabolized (28). In rabbit smooth muscle cells (Fig. 1*A*), PDGF BB (10 ng/ml) stimulated the internalization of DiI-labeled Ac-LDL by 3.7-fold, whereas neither PDGF AA (10–70 ng/ml) nor PDGF AB (10–70 ng/ml) had any effect on the uptake of DiI-labeled Ac-LDL (data not shown). TGF-β_1 (10 ng/ml) up-regulated scavenger receptor activity ~2-fold. The growth factors IGF-I (20 ng/ml) or EGF (10 ng/ml) had little effect on receptor activity when added to cells alone at the concentrations shown here or at concentrations up to 100 ng/ml (data not shown). However, when these growth factors were added in combination, synergistic effects were observed. The four combinations, IGF-I and TGF-β_1, EGF and PDGF BB, TGF-β_1 and PDGF BB, and EGF and TGF-β_1, stimulated receptor activity to a significantly greater extent than that observed with any growth factor alone. The greatest synergistic effect with two growth factors was with EGF and TGF-β_1, in which receptor activity was increased 7.5-fold. An even greater increase in receptor activity (12-fold) was obtained with a combination of the three growth factors EGF, TGF-β_1, and PDGF BB. Addition of IGF-I with the three growth factors had little, if any, additional effect on receptor activity.

In human smooth muscle cells the growth factors also had synergistic effects on scavenger receptor activity (Fig. 1*B*). Whereas IGF-I, EGF, TGF-β_1, and PDGF BB alone had little effect on receptor activity, EGF and TGF-β_1 together had a substantial effect, up-regulating receptor activity approximately 3.4-fold. The addition of either IGF-I or PDGF BB, together with these two growth factors, increased receptor activity up to 7-fold. The addition of a fourth growth factor had no additional stimulatory effect. A similar effect of the combination of growth factors was obtained with a second human aortic

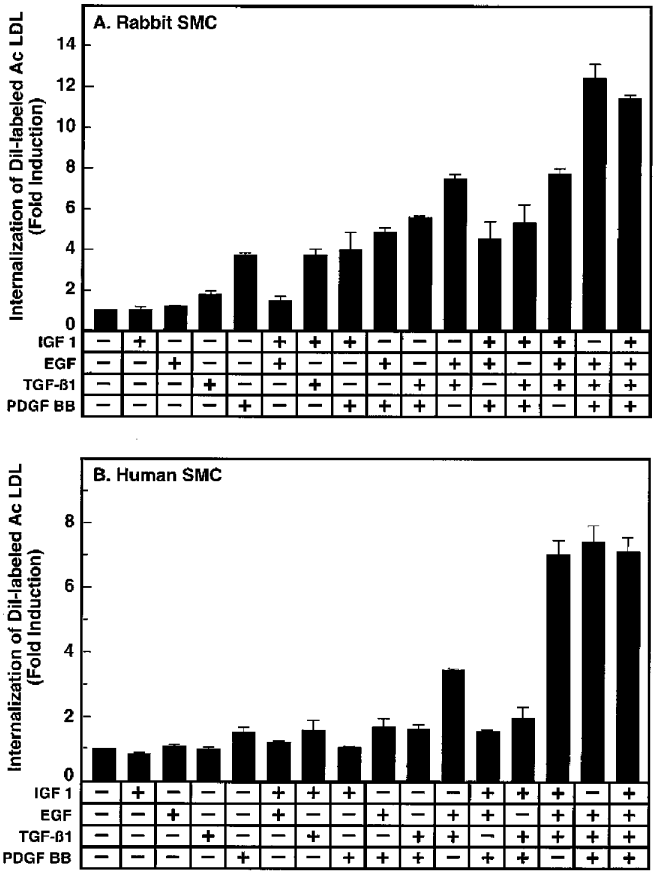

FIG. 1. **Fluorescence-activated cell sorter analysis showing the effect of growth factors on the uptake of DiI-labeled Ac-LDL by rabbit and human smooth muscle cells (*SMC*).** Cells were preincubated in serum-free medium for 16 h at 37 °C with or without EGF (10 ng/ml), PDGF BB (10 ng/ml), TGF-β_1 (10 ng/ml), and IGF-I (20 ng/ml) either alone or in combination. The cells were then incubated with DiI-labeled Ac-LDL (5 μg/ml) for 8 h at 37 °C. After incubation the cells were subjected to FACS analysis as described under "Experimental Procedures." The relative fluorescence intensity, which is proportional to the uptake of DiI-labeled Ac-LDL, was determined for each group of cells. The data are reported as the fold increase in the uptake of DiI-labeled Ac-LDL, which represents the average fluorescent intensity of each group normalized to the fluorescent intensity of the control smooth muscle cells incubated in the absence of growth factors. The data are the mean ± standard deviation (n = 3). In this experiment platelet secretory products (40 μg/ml) stimulated scavenger receptor activity ~4-fold (data not shown). *Panels A* and *B* represent the effect of the growth factors on scavenger receptor activity in rabbit and human smooth muscle cells, respectively.

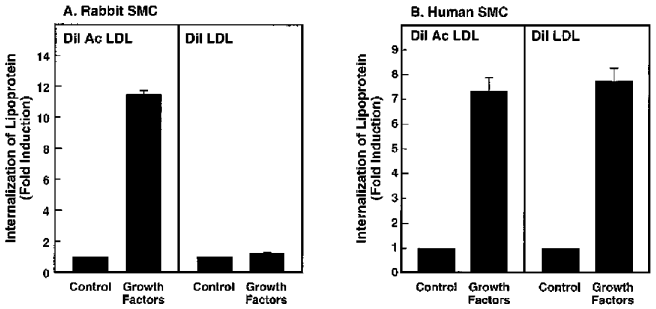

FIG. 2. **Comparison of the effect of growth factors on the internalization of DiI-labeled Ac-LDL and DiI-labeled LDL by rabbit and human smooth muscle cells (*SMC*).** Rabbit (*panel A*) or human (*panel B*) smooth muscle cells were preincubated with medium alone or with medium containing a combination of EGF, PDGF BB, TGF-β_1, and IGF-I for 16 h at 37 °C before assessing their ability to internalize the DiI-labeled lipoproteins (as described in the legend to Fig. 1). Values are the fold increase in the uptake of DiI-labeled Ac-LDL or LDL relative to the internalization observed in cells grown in the absence of growth factors. The data are reported as the mean ± the range for two independent experiments.

smooth muscle cell line (data not shown). The growth factors, therefore, had similar effects on human and rabbit smooth muscle cell scavenger receptor activity, except that PDGF was more potent in the rabbit cells, and IGF-I had more of an effect in the human cells.

The effect of the recombinant growth factors on the internalization of both Ac-LDL and native LDL by rabbit and human smooth muscle cells was examined to determine if the regulation of receptor activity was specific for the scavenger receptor or if LDL receptor activity was also increased. In rabbit smooth muscle cells scavenger receptor activity was stimulated by the mixture of the four growth factors (PDGF BB, EGF, TGF-β_1, and IGF-I), whereas LDL receptor activity was not increased (Fig. 2*A*). In contrast, in human smooth muscle cells, the uptake of both Ac-LDL and native LDL was up-regulated by the combination of the four growth factors (Fig. 2*B*).

Whereas the internalization of DiI-labeled Ac-LDL has been shown to be proportional to the amount of Ac-LDL internalized and degraded in other systems, it was important to determine directly whether the enhanced uptake of DiI-labeled Ac-LDL induced by the growth factors also led to increased lipoprotein metabolism in these studies. For this purpose, the smooth muscle cells were pretreated with the combination of the four growth factors, and the effect on the metabolism of ^{125}I-Ac-LDL was determined. The degradation of ^{125}I-labeled Ac-LDL was enhanced 3.8-fold in human smooth muscle cells incubated with the growth factors at 37 °C for 16 h, and the cellular association of ^{125}I-Ac-LDL, which represents bound and internalized ^{125}I-Ac-LDL, was enhanced 2.9-fold (data not shown). These data therefore confirm the results of the FACS analysis (Fig. 1*B*) and demonstrate growth factor-mediated enhanced internalization and degradation of Ac-LDL by human smooth muscle cells. Similar results were obtained in rabbit smooth muscle cells (data not shown).

Previously we have shown that expression of types I and II scavenger receptor was increased by phorbol ester treatment of rabbit smooth muscle cells (11, 12). To determine whether the enhancement of scavenger receptor activity by these growth factors is due to an induction of types I and II scavenger receptor expression, we performed RNase protection assays to examine scavenger receptor mRNA levels using a probe specific for both the types I and II isoforms. In this particular study scavenger receptor mRNA was not detected in rabbit smooth muscle cells under basal tissue culture conditions (Fig. 3). However, treatment of the smooth muscle cells with the combination of the four growth factors for 16 h increased the expression of both types I and II scavenger receptor mRNA. Similar induction of scavenger receptor mRNA expression was observed in human smooth muscle cells (data not shown).

To determine the mechanism of growth factor stimulation, we first tested the effect of a tyrosine kinase inhibitor on the stimulatory effect of the growth factors on scavenger receptor activity. Incubation of rabbit smooth muscle cells with growth factors led to a 12-fold increase in scavenger receptor activity that was essentially abolished by tyrphostin 47, a tyrosine kinase inhibitor (Table I). Tyrphostin 1, an inactive analog of tyrphostin 47, had little effect. In human smooth muscle cells the growth factor-induced increase in receptor expression was also blocked by tyrphostin 47 (Table I). These data demonstrate that the growth factor-mediated induction of scavenger receptor activity in both rabbit and human smooth muscle cells

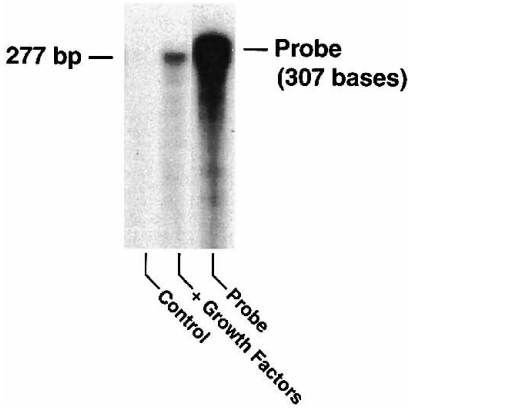

FIG. 3. **RNase protection assay of total RNA from control and growth factor-treated rabbit smooth muscle cells.** Total RNA (10 μg) isolated from the control smooth muscle cells and from smooth muscle cells treated with a mixture of EGF, PDGF BB, TGF-β_1, and IGF-I for 16 h at 37 °C (as described in the legend to Fig. 1) was subjected to RNase protection assay under the conditions described under "Experimental Procedures." *bp*, base pairs.

TABLE I

Effects of the tyrosine kinase inhibitor tyrphostin 47 and the protein kinase C inhibitor MDL.29,152 on the growth factor-mediated regulation of scavenger receptor activity in smooth muscle cells

The cells were preincubated (as described in the legend to Fig. 1) with a mixture of EGF, PDGF BB, IGF-I, and TGF-β_1 alone or together with tyrphostin 47, a tyrosine kinase inhibitor (50 μM), tyrphostin 1, an inactive analog of tyrphostin 47 (50 μM), or MDL.29,152, a protein kinase C inhibitor (100 μM). Receptor activity was then assessed by examining the uptake of DiI-labeled Ac-LDL as described in the legend to Fig. 1. The data are reported as the fold increase in receptor activity (mean ± range for two independent experiments) over the activity observed in the absence of the growth factors.

Treatment	Fold increase in receptor activity	
	Rabbit smooth muscle cells	Human smooth muscle cells
Growth factors alone	11.9 ± 0.5	6.0 ± 0.1
Growth factors + tyrphostin 47	1.9 ± 0.2	1.2 ± 0.1
Growth factors + tyrphostin 1	10.3 ± 0.2	6.1 ± 0.4
Growth factors + MDL.29,152	0.6 ± 0.1	6.6 ± 0.8

requires tyrosine kinase activity.

In some cases tyrosine kinase-mediated signal transduction is associated with protein kinase C activation, and we have shown that protein kinase C is involved in the up-regulation of scavenger receptor activity in rabbit smooth muscle cells (10). For these reasons we examined the effect of protein kinase C inhibitors on growth factor-induced up-regulation of scavenger receptor activity. Coincubation of rabbit smooth muscle cells with growth factors together with MDL.29,152 (50 or 100 μM), a protein kinase C inhibitor (24), abolished the stimulatory effect of the growth factors on scavenger receptor activity (Table I). This result suggests that protein kinase C functions in the signal transduction pathway leading to receptor up-regulation in rabbit smooth muscle cells. In contrast, MDL.29,152 (at concentrations up to 150 μM) did not block stimulation of scavenger receptor activity by growth factors in human smooth muscle cells (Table I). The MDL.29,152 (50 μM) did, however, block the phorbol ester-induced stimulation of scavenger receptor activity in human smooth muscle cells and blocked the phorbol ester-induced differentiation (adhesion) of the human monocyte cell line THP-1, demonstrating that the inhibitor was active in human cells (data not shown). The data suggest differences between the mechanism of regulation of scavenger receptor activity by growth factors in human and rabbit smooth muscle cells.

TABLE II

Quantitation of TGF-β_1, PDGF, EGF, and IGF-I in human platelet secretory products

Western blot analysis was performed under the conditions described under "Experimental Procedures." The amount of each growth factor in platelet secretory products was estimated by densitometric scanning of Western blots using recombinant growth factors run on the same gel as standards.

Growth factors	Concentration in platelet secretory products
	ng/μg
TGF-β_1	0.35
PDGF	0.11
EGF	Undetectable (<0.03)
IGF-I	Undetectable (<0.03)

Our data clearly demonstrate that PDGF BB, EGF, IGF-I, and TGF-β_1 in combination synergistically stimulate scavenger receptor activity in smooth muscle cells. We next performed experiments to determine whether these growth factors are the components in our preparation of platelet secretory products responsible for stimulation of scavenger receptor activity. We first estimated the amount of the four growth factors present in platelet secretory products using Western blot analysis. The platelet secretory products contained approximately 0.35 ng of TGF-β_1, 0.11 ng of PDGF, and less than 0.03 ng of EGF and IGF-I/μg (Table II). Therefore, 40 μg of platelet secretory products, the level that gave maximum stimulation of scavenger receptor activity, contained ~4.4 ng of PDGF, ~14 ng of TGF-β_1, and less than 1 ng each of EGF and IGF-I. We next performed neutralization experiments to determine whether these growth factors play any functional role in mediating the stimulatory effect of platelet secretory products on scavenger receptor activity. As shown in Fig. 4, an anti-PDGF antibody, used at a concentration sufficient to block the effect of recombinant PDGF BB, did not block the stimulatory effect of platelet secretory products on scavenger receptor activity. Antibodies specific for EGF and IGF-I also had little effect. Anti-TGF-β_1 antibody, on the other hand, blocked the stimulatory effect of platelet secretory products by ~70%, suggesting that TGF-β_1 is one of the active components in platelet secretory products. However, approximately 30% of the stimulatory activity remained, and the addition of anti-PDGF and anti-TGF-β_1 together or the addition of a mixture of the four antibodies did not result in a greater inhibition of the stimulatory effect than that observed with anti-TGF-β_1 alone. These data indicate that TGF-β_1 is responsible for 70% of the stimulatory effect of platelet secretory products on scavenger receptor activity and that the remaining 30% of the activity is not mediated by the growth factors tested.

Two additional experiments support the conclusion that PDGF is not the active component in platelet secretory products. First, when 4.4 ng of recombinant PDGF BB (the amount of PDGF estimated to be present in 40 μg of platelet secretory products, which gives maximum stimulation of receptor activity) was used to treat the rabbit smooth muscle cells, it failed to stimulate scavenger receptor activity, indicating that the amount of PDGF in 40 μg of platelet secretory products is too low to be responsible for the effect of platelet secretory products in our *in vitro* studies (see Fig. 5). Second, an antagonist to PDGF (Trapidil, 100 μg/ml) which blocked the effect of recombinant PDGF (50 ng/ml) (33) failed to block the stimulatory effect of platelet secretory products on scavenger receptor activity (data not shown). We also tested the ability of recombinant TGF-β_1 (14 ng) and PDGF (4.4 ng) (the amounts present

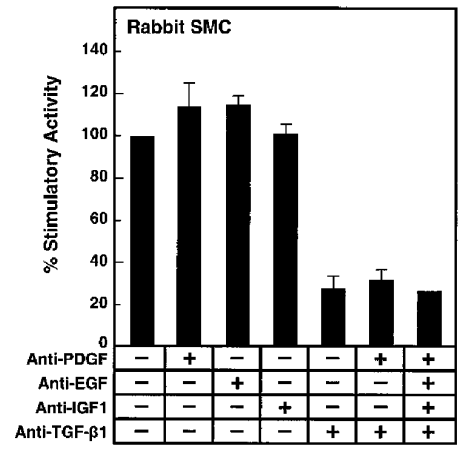

FIG. 4. **Ability of blocking antibodies to neutralize the stimulatory effect of platelet secretory products on scavenger receptor activity.** Smooth muscle cells (*SMC*) were preincubated with 40 μg of platelet secretory products in the absence or presence of blocking antibodies against PDGF (33 μg/ml), EGF (13 μg/ml), IGF-I (10 μg/ml), and TGF-β_1 (8 μg/ml) for 16 h at 37 °C and then assayed for their ability to internalize DiI-labeled Ac-LDL. The data are reported as the mean ± the range for two independent experiments.

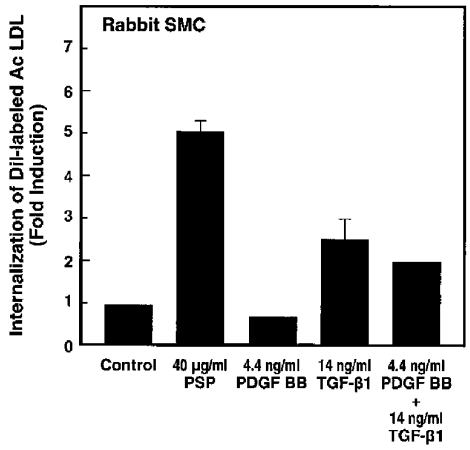

FIG. 5. **Effect of platelet secretory products, PDGF BB, and TGF-β_1 alone or in combination on scavenger receptor activity in rabbit smooth muscle cells (*SMC*).** Smooth muscle cells were pretreated with 40 μg/ml of platelet secretory products (*PSP*) or with either recombinant PDGF BB or TGF-β_1 alone or together, at the concentrations indicated, for 16 h at 37 °C and then assayed as described in Fig. 1.

in the maximally active concentration of platelet secretory products) alone and together to up-regulate scavenger receptor activity (Fig. 5). Neither TGF-β_1 nor PDGF alone or in combination increased receptor activity to the level obtained with the platelet secretory products, again suggesting that there are other factors besides TGF-β_1 in platelet secretory products which can increase receptor activity either alone or in combination with TGF-β_1.

DISCUSSION

We have shown previously that types I and II scavenger receptor activity in rabbit smooth muscle cells can be up-regulated by phorbol esters, platelet secretory products in serum, and secretion products from activated platelets (10–12). In addition, it has been reported that tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) increase scavenger receptor activity in rabbit smooth muscle cells (17). In the current studies we demonstrated that growth factors stimulate scavenger receptor activity in both human and rabbit smooth muscle cells. PDGF BB and TGF-β_1 increased scavenger receptor activity approximately 4- and 2-fold, respectively, in rabbit smooth muscle cells but not in human smooth muscle cells. EGF or IGF-I, when used alone, had little effect on scavenger receptor activity in either human or rabbit smooth muscle cells. However, when incubated with the cells in combination, these growth factors showed synergistic effects on scavenger receptor activity. In rabbit smooth muscle cells PDGF BB and either EGF or TGF-β_1, and EGF together with TGF-β_1, had synergistic effects in raising scavenger receptor activity, and the addition of all three growth factors together increased receptor activity 12-fold. In human smooth muscle cells EGF and TGF-β_1 clearly had a synergistic effect on the induction of scavenger receptor activity, and the addition of either IGF-I or PDGF BB together with these two growth factors increased receptor activity up to 7-fold. We demonstrated that the increase in scavenger receptor activity in both rabbit and human smooth muscle cells by the growth factors is correlated with an increase in type I and II scavenger receptor mRNA expression. We have shown previously that the scavenger receptor expressed by the smooth muscle cells had properties essentially identical to those of the bovine macrophage types I and II scavenger receptors (11, 12).

In rabbit smooth muscle cells the growth factors specifically increased scavenger receptor activity with no effect on LDL receptor activity. In striking contrast to this, in human smooth muscle cells both scavenger receptor and LDL receptor activities were increased. Other investigators have demonstrated previously that growth factors (such as TGF-β and PDGF) stimulate LDL receptor activity in human smooth muscle cells (34, 35). The reasons for the difference in regulation of LDL receptor activity in the rabbit and human smooth muscle cells remain to be determined; however, they could be related to differences in the signal transduction pathways in the rabbit and human cells or to differences in transcription factor binding sites in the human and rabbit LDL receptor genes.

Our results concerning regulation of scavenger receptor activity by growth factors differ slightly from those of Inaba *et al.* (36, 37), in which they observed a stimulatory effect of PDGF BB or EGF alone on scavenger receptor activity in human smooth muscle cells. We noted a slight stimulatory effect of PDGF BB alone but did not see an effect of EGF alone in two lines of human smooth muscle cells. The difference in these results may be related to the difference in experimental conditions or may simply reflect differences in the properties of various lines of human smooth muscle cells.

Whereas scavenger receptor activity in smooth muscle cells is regulated over a wide range, in differentiated macrophages scavenger receptor activity can be regulated in a narrow range by various growth factors and cytokines. Incubation of macrophages with macrophage colony-stimulating factor results in an increase in scavenger receptor activity (38), whereas incubation of macrophages with TGF-β_1 (39), IFN-γ (40–42), and TNF-α (43) reduces receptor activity. The reason for the differential effects of IFN-γ, TNF-α, and TGF-β_1 on scavenger receptor activity in smooth muscle cells and macrophages is unknown. The regulation of scavenger receptor activity in smooth muscle cells and macrophages, however, is fundamentally different. In macrophages scavenger receptor expression is constitutively stimulated, and they express a high level of receptor activity. In smooth muscle cells scavenger receptor expression is low in the absence of stimulation.

As we have shown, platelet secretory products regulate scavenger receptor activity in smooth muscle cells. Platelet secretory products can also affect scavenger receptor activity in

macrophages. It has been reported that activated platelets secrete a protein-like factor that stimulates scavenger receptor activity in macrophages (44) and that platelets secrete a ligand for the scavenger receptor which competitively inhibits the binding of modified LDL to the scavenger receptor (45, 46). Several platelet secretory products, including serotonin, fibrinogen, fibronectin, and PDGF, inhibit scavenger receptor activity in human monocyte-derived macrophages (47). The current data demonstrating that growth factors present in platelet secretory products stimulate scavenger receptor activity in smooth muscle cells suggest that platelets in atherosclerotic lesions could modulate lipid accumulation in both smooth muscle cells and macrophages.

The recent studies of Li *et al.* (17) demonstrate that after balloon injury, the aorta smooth muscle cells in the neointima of hypercholesterolemic rabbits express scavenger receptors, whereas smooth muscle cells in the media do not. These data clearly demonstrate that scavenger receptor expression is upregulated in smooth muscle cells in atherosclerotic lesions. The factors leading to scavenger receptor regulation in smooth muscle cells *in vivo* are unknown. However, following deendothelialization induced by balloon injury, extensive platelet deposition would occur, making growth factors secreted by activated platelets available to regulate scavenger receptor activity (48–50). In fact, platelets are not the only source of growth factors in the atherosclerotic lesion. Injured endothelial cells, activated macrophages, and smooth muscle cells themselves all secret growth factors that we have demonstrated to stimulate scavenger receptor activity in smooth muscle cells (22). It has been shown that the expression of PDGF, IGF-I, TGF-β_1, and EGF is increased in atherosclerotic lesions (19–23, 51, 52), suggesting that they could play a role in regulation of scavenger receptor activity in smooth muscle cells. Although platelets are not an early component of atherosclerotic lesions, in the absence of deendothelialization, macrophages are. In fact, macrophages are the primary component of early fatty streak lesions, present initially in vast excess to the migrating and proliferating smooth muscle cells. These macrophages are known to secret PDGF, EGF, IGF-I, and TGF-β_1, as well as IFN-γ and TNF-α (22), all of which regulate scavenger receptor activity in smooth muscle cells *in vitro*. We propose that these growth factors and cytokines are responsible for the regulation of scavenger receptor activity in atherosclerotic lesions.

Thus far, little is known about the mechanisms of stimulation of scavenger receptor activity in macrophages and smooth muscle cells. Wu *et al.* (53) have shown that scavenger receptor expression in human monocyte-derived macrophages is regulated via a signal transduction pathway involving ras, Ap1 (c-jun and junB), and ets domain proteins. Our preliminary data show that stimulation of scavenger receptor activity by growth factors requires cellular tyrosine kinase activity in both human and rabbit smooth muscle cells. It has been shown that the binding of EGF, IGF-I, and PDGF to their corresponding receptors activates receptor-associated tyrosine kinase activity followed by a host of intracellular signal events including activation of the ras-mitogen-activated protein kinase pathway, which in turn can activate jun family proteins (54). It will be important to determine whether or not the ras-mitogen-activated protein kinase pathway and jun proteins are involved in the growth factor-induced stimulation of scavenger receptor activity in smooth muscle cells.

It has been proposed that modified LDL is the atherogenic ligand for types I and II scavenger receptor on macrophages which causes the massive accumulation of lipid and foam cell formation (5–8, 55–58). We have demonstrated that smooth muscle cells express types I and II scavenger receptors that bind, internalize, and degrade modified LDL (10–12), suggesting that the scavenger receptor-mediated uptake of modified LDL by smooth muscle cells may also lead to lipid accumulation and foam cell formation *in vivo* as proposed in macrophages. The data in the current study suggest that growth factors secreted by cells in developing atherosclerotic lesions could stimulate scavenger receptor activity in smooth muscle cells and contribute to lipid accumulation and foam cell formation.

Acknowledgments—We thank Annabelle Friera and James McGuire for excellent technical assistance; Sherrill Peterson, Kerry Humphrey, and Don Haumant for manuscript preparation; Gary Howard for editorial support; and Amy Corder, John Carroll, and Stephen Gonzales for graphics. FACS analysis was performed by Kris Kavanau at the University of California, San Francisco, Laboratory for Cell Analysis.

REFERENCES

1. Ross, R. (1986) *N. Engl. J. Med.* **314,** 488–500
2. Brown, M. S., and Goldstein, J. L. (1983) *Annu. Rev. Biochem.* **52,** 223–261
3. Fowler, S., Shio, H., and Haley, N. J. (1979) *Lab. Invest.* **41,** 372–378
4. Schaffner, T., Taylor, K., Bartucci, E. J., Fischer-Dzoga, K., Beeson, J. H., Glagov, S., and Wissler, R. W. (1980) *Am. J. Pathol.* **100,** 57–80
5. Witztum, J. L., and Steinberg, D. (1991) *J. Clin. Invest.* **88,** 1785–1792
6. Haberland, M. E., and Fogelman, A. M. (1987) *Am. Heart J.* **113,** 573–577
7. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., and Witztum, J. L. (1989) *N. Engl. J. Med.* **320,** 915–924
8. Goldstein, J. L., Ho, Y. K., Basu, S. K., and Brown, M. S. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 333–337
9. Pitas, R. E., Gong, Q., McGuire, J., Friera, A., and Mietus-Snyder, M. (1995) *Proc. Mexican Biochem. Soc. Symp.*, in press
10. Pitas, R. E. (1990) *J. Biol. Chem.* **265,** 12722–12727
11. Pitas, R. E., Friera, A., McGuire, J., and Dejager, S. (1992) *Arterioscler. Thromb.* **12,** 1235–1244
12. Dejager, S., Mietus-Snyder, M., and Pitas, R. E. (1993) *Arterioscler. Thromb.* **13,** 371–378
13. Gerrity, R. G. (1981) *Am. J. Pathol.* **103,** 181–190
14. Fogelman, A. M., Haberland, M. E., Seager, J., Hokom, M., and Edwards, P. A. (1981) *J. Lipid Res.* **22,** 1131–1141
15. Jaakkola, O., and Nikkari, T. (1990) *Am. J. Pathol.* **137,** 457–465
16. Inaba, T., Yamada, N., Gotoda, T., Shimano, H., Shimada, M., Momomura, K., Kadowaki, T., Motoyoshi, K., Tsukada, T., Morisaki, N., Saito, Y., Yoshida, S., Takaku, F., and Yazaki, Y. (1992) *J. Biol. Chem.* **267,** 5693–5699
17. Li, H., Freeman, M. W., and Libby, P. (1995) *J. Clin. Invest.* **95,** 122–133
18. Bickel, P. E., and Freeman, M. W. (1992) *J. Clin. Invest.* **90,** 1450–1457
19. Hajjar, D. P., and Pomerantz, K. B. (1992) *FASEB J.* **6,** 2933–2941
20. Munro, J. M., and Cotran, R. S. (1988) *Lab. Invest.* **58,** 249–260
21. Clinton, S. K., and Libby, P. (1992) *Arch. Pathol. Lab. Med.* **116,** 1292–1300
22. Ross, R. (1993) *Nature* **362,** 801–809
23. Kawakami, M., and Kuroki, M. (1993) *Nippon Rinsho* **51,** 2010–2015
24. Akeson, A. L., Schroeder, K., Woods, C., Schmidt, C. J., and Jones, W. D. (1991) *J. Lipid Res.* **32,** 1699–1707
25. Pitas, R. E., Innerarity, T. L., and Mahley, R. W. (1980) *J. Biol. Chem.* **255,** 5454–5460
26. Bilheimer, D. W., Eisenberg, S., and Levy, R. I. (1972) *Biochim. Biophys. Acta* **260,** 212–221
27. Innerarity, T. L., Pitas, R. E., and Mahley, R. W. (1986) *Methods Enzymol.* **129,** 542–565
28. Pitas, R. E., Innerarity, T. L., Weinstein, J. N., and Mahley, R. W. (1981) *Arteriosclerosis* **1,** 177–185
29. Basu, S. K., Goldstein, J. L., Anderson, R. G. W., and Brown, M. S. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73,** 3178–3182
30. Goldstein, J. L., Basu, S. K., and Brown, M. S. (1983) *Methods Enzymol.* **98,** 241–260
31. Dejager, S., Mietus-Snyder, M., Friera, A., and Pitas, R. E. (1993) *J. Clin. Invest.* **92,** 894–902
32. Holmsen, H. (1989) *Ann. Med.* **21,** 23–30
33. Kuratsu, J.-I., and Ushio, Y. (1990) *J. Neurosurg.* **73,** 436–440
34. Nicholson, A. C., and Hajjar, D. P. (1992) *J. Biol. Chem.* **267,** 25982–25987
35. Roth, M., Emmons, L. R., Perruchoud, A., and Block, L. H. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 1888–1892
36. Inaba, T., Gotoda, T., Shimano, H., Shimada, M., Harada, K., Kozaki, K., Watanabe, Y., Hoh, E., Motoyoshi, K., Yazaki, Y., and Yamada, N. (1992) *J. Biol. Chem.* **267,** 13107–13112
37. Inaba, T., Gotoda, T., Harada, K., Shimada, M., Ohsuga, J.-I., Ishibashi, S., Yazaki, Y., and Yamada, N. (1995) *J. Clin. Invest.* **95,** 1133–1139
38. Clinton, S. K., Underwood, R., Hayes, L., Sherman, M. L., Kufe, D. W., and Libby, P. (1992) *Am. J. Pathol.* **140,** 301–316
39. Bottalico, L. A., Wager, R. E., Agellon, L. B., Assoian, R. K., and Tabas, I. (1991) *J. Biol. Chem.* **266,** 22866–22871
40. Kraemer, F. B., Tavangar, K., Gandjei, R. K., Kirlew, K., and Behr, S. R. (1990) *Arteriosclerosis* **10,** 8–16
41. Geng, Y.-J., and Hansson, G. K. (1992) *J. Clin. Invest.* **89,** 1322–1330
42. Fong, L. G., Fong, T. A. T., and Cooper, A. D. (1990) *J. Biol. Chem.* **265,** 11751–11760
43. van Lenten, B. J., and Fogelman, A. M. (1992) *J. Immunol.* **148,** 112–116
44. Fuhrman, B., Brook, G. J., and Aviram, M. (1991) *J. Lipid Res.* **32,** 1113–1123

45. Phillips, D. R., Arnold, K., and Innerarity, T. L. (1985) *Nature* **316,** 746–748
46. Mas-Oliva, J., Arnold, K. S., Wagner, W. D., Phillips, D. R., Pitas, R. E., and Innerarity, T. L. (1994) *J. Biol. Chem.* **269,** 10177–10183
47. Aviram, M. (1989) *Metabolism* **38,** 425–430
48. Gutstein, W. H., Farrell, G. A., and Armellini, C. (1973) *Lab. Invest.* **29,** 134–149
49. Lewis, J. C., and Kottke, B. A. (1977) *Science* **196,** 1007–1009
50. Ross, R. (1981) *Arteriosclerosis* **1,** 293–311
51. Tamamoto, T., Toda, T., Shimajiri, S., Kiyuna, M., Shingaki, Y., Nakashima, Y., and Takei, H. (1994) *Rinsho Byori* **42,** 971–976
52. Li, Z., Alavi, M. Z., and Moore, S. (1994) *Int. J. Exp. Pathol.* **75,** 169–177
53. Wu, H., Moulton, K., Horvai, A., Parik, S., and Glass, C. K. (1994) *Mol. Cell. Biol.* **14,** 2129–2139
54. Marshall, C. J. (1995) *Cell* **80,** 179–185
55. Mahley, R. W., Weisgraber, K. H., Innerarity, T. L., and Windmueller, H. G. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 1746–1750
56. Fogelman, A. M., Shechter, I., Seager, J., Hokom, M., Child, J. S., and Edwards, P. A. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 2214–2218
57. Mahley, R. W., Innerarity, T. L., Weisgraber, K. H., and Oh, S. Y. (1979) *J. Clin. Invest.* **64,** 743–750
58. Freeman, M., Ekkel, Y., Rohrer, L., Penman, M., Freedman, N. J., Chisolm, G. M., and Krieger, M. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 4931–4935