Transforming Growth Factor-β1 Inhibits Scavenger Receptor Activity in THP-1 Human Macrophages*

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The macrophage scavenger receptor, a 220-kDa trimeric membrane glycoprotein, mediates the internalization of modified forms of low density lipoprotein (LDL) such as acetyl-LDL and oxidized-LDL and thus is likely to play a key role in atheroma macrophage foam cell formation. In addition, recent evidence suggests that the scavenger receptor may be an important macrophage binding site for lipopolysaccharide involved in lipopolysaccharide scavenging by macrophages. However, little is known about the regulation of this important receptor. We now report that the induction of scavenger receptor activity (as measured by acetyl-LDL stimulation of intracellular cholesterol esterification) seen in phorbol ester-differentiated THP-1 human macrophages was completely suppressed to the level seen in undifferentiated THP-1 monocytes by picomolar concentrations of transforming growth factor-β1 (TGF-β1). 125I-Acetyl-LDL degradation was inhibited in a dose-dependent manner by TGF-β1, with maximal inhibition (~70%) occurring at 24 pM TGF-β1. Scatchard analysis revealed that TGF-β1 treatment resulted in a ~2-fold decrease in receptor number, and Northern blot analysis of RNA isolated from differentiated THP-1 macrophages demonstrated ~2-fold less scavenger receptor mRNA in TGF-β1-treated monocytes compared with that in macrophages not treated with TGF-β1. Since TGF-β1 is thought to be present in both atherosclerotic and inflammatory lesions, the above findings may have physiological relevance regarding the regulation of atheroma foam cell formation and/or the regulation of lipopolysaccharide clearance by macrophages.

Macrophages are prominent components of both atherosclerotic (1-3) and inflammatory lesions (4). In atherosclerotic lesions, macrophages accumulate large amounts of cholesterol ester and are termed “foam cells” (5). Foam cells form when macrophages internalize lipoproteins, an event which leads to the stimulation of the intracellular cholesterol esterification enzyme, acyl-CoA:cholesterol acyltransferase (ACAT) (5). A major lipoprotein internalization pathway involved in macrophage ACAT stimulation and foam cell formation involves the uptake of modified forms of LDL (e.g. acetylated or oxidized LDL) by a protein called the scavenger receptor (5).

Two types of scavenger receptors have been described. The “type II” receptor is a 453-amino acid plasma membrane trimeric glycoprotein whose extracellular C-terminal region includes a α-helical coiled-coil domain, a collagen-like domain, and a 110-amino-acid terminal cysteine-rich region (6). The “type II” receptor is identical to the type I receptor except for the absence of the C-terminal cysteine-rich region (6). There is growing evidence that the macrophage scavenger receptor is involved in foam cell formation in vivo (8-10). In addition, the macrophage scavenger receptor is probably involved in the in vivo “scavenging” or clearance of other physiologically important molecules (11). For instance, the scavenger receptor binds bacterial LPS (without a cytokine response) and may be involved in the clearance of LPS from tissues (12). Thus, the macrophage scavenger receptor has potentially important functions in a wide variety of physiological and pathophysiological conditions.

Despite the obvious importance of the macrophage scavenger receptor and the finding that the expression of this receptor on macrophages in human atherosclerotic lesions is not uniform (10) (suggesting in vivo regulation of the receptor), very little is known about the factors involved in macrophage scavenger receptor regulation. In contrast to LDL receptor regulation, increased intracellular cholesterol does not down-regulate the scavenger receptor (5). Rather, scavenger receptor activity and mRNA appear to be up-regulated by monocyte-to-macrophage differentiation as occurs when human peripheral blood monocytes differentiated in cell culture (13) or when human THP-1 monocytes differentiate as a result of phorbol ester (PMA) treatment (14, 15). In addition, platelet secretory products appear to inhibit macrophage scavenger receptor activity (16, 17), although the exact mediators of this effect have not yet been identified.

Given the potential importance of intrinsic monocyte/macrophage factors and extrinsic platelet factors in the regulation of macrophage scavenger receptor activity and the fact that...
transforming growth factor-β1 (TGF-β1) is an important secretory product of both macrophages and platelets (18-20), we explored the effect of TGF-β1 on macrophage scavenger receptor activity. TGF-β1 is a 25-kDa dimeric protein that can both stimulate and inhibit the growth and differentiation of many different cell types (18, 19). The specific effects of TGF-β1 depend upon many factors, including the cell type and the absence or presence of other growth factors (18, 19).

In this report, we show that TGF-β1 inhibits scavenger receptor activity in THP-1 human monocyte/macrophages, at least in part by decreasing scavenger receptor mRNA levels in these cells.

**EXPERIMENTAL PROCEDURES**

**Cells—Suspensions of THP-1 cells (American Type Culture Collection) were maintained in RPMI-1640 supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 5 × 10⁻⁵ M β-mercaptoethanol, and 10% fetal bovine serum. For all experiments, the cells were preincubated for 24 h in medium in which the fetal bovine serum was replaced with 10% lipoprotein-deficient serum (RPMI-LPDS). The cells were then resuspended in RPMI-LPDS plus or minus 0.16-1.0 μM phorbol 12-myristate 13-acetate (PMA). Signa was dissolved in aceton (final concentration of aceton in media = 0.1%) and plated at a density of 5 × 10⁴ cells/ml into culture tissue dishes (Falcon or Corning). The cells were incubated for 2-3 days in this medium in the presence of human recombinant or porcine platelet TGF-β1 (Geno/BRL or R & D Systems, respectively).

**Lipoproteins—LDL (density, 1.020-1.063 g/ml) was isolated by preparative ultracentrifugation. Acetyl-LDL was prepared by reaction with acetic anhydride as described by Basu et al. (20). The lipoproteins were labeled with 125I (Du Pont-New England Nuclear, carrier-free) using lactoperoxidase as described previously (21). LDL lipoproteins were 95% trichloroacetic acid-precipitable, 95% chloroform-nonextractable, and had a specific activity of 200-400 cpm/ng, and, by SDS-polyacrylamide gel electrophoresis, had all of the label in apoB100.

All radioactively labeled lipoproteins were used within 3 weeks of the labeling procedure.

**Whole Cell ACAT and 125I-Lipoprotein Binding and Degradation Assays—ACAT activity in intact cells was measured by the [14C]oleate pulse method as described (22). 125I-Lipoprotein binding was assayed by determining cell-associated [125I]cpm after a 2-h, 4°C incubation of cells with 125I-lipoproteins (23). 125I-Lipoprotein degradation was assayed by measuring trichloroacetic acid-soluble, chloroform-nonextractable [125I]cpm in the media of cells incubated with the labeled lipoproteins for 6 h at 37°C (22). For both the binding and the degradation measurements, assays in the presence of excess unlabeled lipoproteins were conducted to determine nonspecific values. Specific binding or degradation was then calculated by subtracting these nonspecific values from the total values described above.

**Northern Blot Analysis of Poly(A)+ RNA from THP-1 Cells—**Total RNA was isolated from THP-1 cells according to the procedure of Chomczynski and Sacchi (23) and subjected to Northern blot analysis as described previously (24). RNA was normalized by both loading equal masses of total RNA and by using a random-primed probe to glyceraldehyde-3-phosphate dehydrogenase. The XbaI-SphI fragment of plasmid pBR32 (6) (donated by Dr. Monty Krieger, Massachusetts Institute of Technology) was radiolabeled with 32P using a random priming method (23) and was hybridized to the membrane at 42°C in the presence of 40% formamide. The membrane was washed with 0.5 × SSC containing 0.1% SDS at 55°C and exposed to an x-ray film for 2 days in the presence of an intensifying screen at -80°C. The relative intensities of the mRNA bands were determined by densitometric scanning of the autoradiograms using a Molecular Dynamics Computing Densitometer (model 300A) with Image-Quant software.

**RESULTS**

**Effect of TGF-β1 on the Induction of Acetyl-LDL-stimulated ACAT Activity in THP-1 Macrophages—**PMA-mediated differentiation of THP-1 monocytes leads to an induction of scavenger receptor activity (as well as a repression of LDL receptor activity) (15). An important functional assay for scavenger receptor activity is the stimulation of the intracellular cholesterol-esterifying enzyme, ACAT, by a lipoprotein ligand for the receptor (e.g., acetyl-LDL) (5). Fig. 1 shows ACAT activity in undifferentiated THP-1 monocytes (CON), PMA-differentiated THP-1 macrophages (+ PMA), and THP-1 macrophages that were PMA-differentiated in the presence of TGF-β1 (+ PMA + TGF-β1). Compared with ACAT activity in the absence of lipoproteins (open bars), LDL-stimulated ACAT activity (diagonally hatched bars) was relatively high in undifferentiated monocytes but much lower in PMA-differentiated macrophages (consistent with previous data (Ref. 15)), and treatment with TGF-β1 did not alter the repression of LDL-induced ACAT activity in the differentiated macrophages. In contrast, acetyl-LDL-stimulated ACAT activity (cross-hatched bars) was very low in the monocytes and much higher in the differentiated macrophages (as reported (Ref. 15)). Furthermore, when TGF-β1 was added during the period of differentiation, the level of acetyl-LDL-stimulated ACAT activity in the differentiated macrophages was no higher than that seen in the undifferentiated monocytes. TGF-β1-treated cells remained adherent and morphologically similar to differentiated macrophages treated with PMA alone, and there was no sign of general cellular toxicity (no decrease in protein content or cell number and no trypan blue uptake). Thus, the induction of acetyl-LDL-stimulated ACAT activity in differentiating THP-1 monocyte/macrophages was inhibited by TGF-β1.

**Effect of TGF-β1 on 125I-Acetyl-LDL Degradation in and Binding to THP-1 Macrophages—**As noted above, lipoprotein uptake and degradation by cells is necessary for ACAT stimulation to occur (5). To determine if the block in induction of acetyl-LDL-stimulated ACAT activity was a result of a corresponding decrease in acetyl-LDL degradation by the macrophages, THP-1 cells were differentiated for 2 days with PMA in the absence or presence of increasing concentrations of TGF-β1, and then, on the third day, specific 125I-acetyl-LDL degradation by the macrophages. This decrease was dependent upon the dose of TGF-β1 administered, with the maximal
To determine the timing of the TGF-β1 inhibitory effect on scavenger receptor activity in THP-1 monocyte/macrophages, the experiments shown in Fig. 3 were conducted. In the experiment shown in Fig. 3A, THP-1 cells were differentiated with PMA for 2 days, and a 6-h 125I-acetyl-LDL degradation assay was conducted on the 3rd day. The data shown by the left bar (labeled None) depicts the effect of adding no TGF-β1 during the incubation or assay period. The effect of adding TGF-β1 during both the differentiation period and during the 6-h assay period is shown by the data in the middle bar (labeled Days 1, 2, 3); a 3-fold inhibition of 125I-acetyl-LDL degradation was observed. If TGF-β1 was added only during the 6-h assay period on the 3rd day (right bar, Days 3), no inhibitory effect was seen. Other experiments revealed that if TGF-β1 was added during the differentiation period but not during the assay, 2–3-fold inhibition of 125I-acetyl-LDL was observed (Figs. 2 and 3B and other data not shown). Thus, TGF-β1-mediated inhibition of 125I-acetyl-LDL degradation by differentiating THP-1 monocyte/macrophages required that the TGF-β1 be present during the differentiation period but not during the assay period. The latter point indicates that TGF-β1 is not a competitive inhibitor of 125I-acetyl-LDL uptake by the cells, as has been proposed for another platelet secretory product (16).

All of the experiments reported to this point have demonstrated a scavenger receptor inhibitory effect of TGF-β1 added...
during the period of PMA-mediated differentiation of THP-1 cells (i.e., during the period of scavenger receptor induction). In the experiment shown in Fig. 3B, we determined whether TGF-β1 could inhibit scavenger receptor activity after the activity had been induced in THP-1 macrophages. In this experiment, THP-1 cells were incubated with PMA for 2 days to induce scavenger receptor activity in the cells. The PMA-containing medium was then removed from the differentiated macrophages, and the cells were then incubated for an additional 2 days in PMA-free medium alone (left bar) or containing TGF-β1 (right bar). On the 5th day, the cells were assayed for 125I-acetyl-LDL degradation (in the absence of TGF-β1). The data clearly show that TGF-β1 added after the period of scavenger receptor induction led to a 3-fold inhibition of 125I-acetyl-LDL degradation. Thus, TGF-β1 inhibited both the induction of scavenger receptor activity in differentiating THP-1 cells as well as previously induced scavenger receptor activity in differentiated THP-1 macrophages.

We next sought to define the step in the internalization/degradative pathway of acetyl-LDL that was blocked by TGF-β1. The data in Fig. 4A show a 4°C 125I-acetyl-LDL specific binding experiment in control (closed circles) and TGF-β1-treated (open circles) cells. Scatchard analysis of these data (Fig. 4B) yielded linear plots and revealed a 2-fold decrease in receptor number (Bmax) as well as a decrease in receptor affinity (Fig. 4B, inset) in TGF-β1-treated cells. Since all of our degradation experiments used a saturating concentration of 125I-acetyl-LDL (25 µg/ml) and since the inhibition of 125I-acetyl-LDL degradation (~70%) was similar to the decrease in receptor number (~50%), we conclude that most of the TGF-β1-mediated decrease in 125I-acetyl-LDL degradation observed in our experiments could be explained by a decrease in cell surface scavenger receptor number.

Northern Blot Analysis of RNA from Control and TGF-β1-treated THP-1 Cells—To determine if the decrease in scavenger receptor number by TGF-β1 was mediated at the mRNA level, Northern blot analysis was performed on total RNA isolated from THP-1 macrophages differentiated by PMA in the absence or presence of TGF-β1. A cDNA probe which recognizes the mRNA of both type I and II scavenger receptors (6) was used for this experiment. Aliquots of the cells used for this experiment were also assayed for specific 125I-acetyl-LDL surface binding and degradation (Fig. 5). In this experiment, TGF-β1 treatment resulted in a 2.0-fold decrease in specific 125I-acetyl-LDL binding and a 2.9-fold decrease in specific 125I-acetyl-LDL degradation (Fig. 5, first two pairs of bars, respectively). In the absence of TGF-β1, prominent scavenger receptor mRNA bands (migrating between 28 and 18 S RNA) were observed on the Northern blot (Fig. 5, inset, SCAV (+)), as had been previously reported (6). (The appearance of two RNA bands was also found by Krieger and co-workers (6); the significance of the two bands is not yet clear.) Treatment with TGF-β1 resulted in a decrease in scavenger receptor mRNA (Fig. 5, inset, SCAV (+)), which, by densitometric analysis of the autoradiograms, was a 2.2-fold decrease (same for both bands). (As expected (Ref. 6), little or no message for the scavenger receptor was detected in THP-1 cells before differentiation by PMA treatment (data not shown).) These Northern blot data were verified in a separate experiment using poly(A)selective RNA from untreated and TGF-β1-treated THP-1 macrophages (data not shown). Thus, the 2.0-fold decrease in scavenger receptor number by TGF-β1 treatment (Fig. 4) likely results from a 2-fold decrease in scavenger receptor mRNA levels.

**DISCUSSION**

Evidence is rapidly accumulating that the macrophage scavenger receptor may play several critically important physiological and pathophysiological roles, including the generation of macrophage foam cells in atherosclerosis (8–10), LPS clearance during Gram-negative bacterial sepsis (12), and macrophage "scavenging" of other toxic molecules (11). Furthermore, there is evidence for in vivo regulation of the receptor since the expression of scavenger receptor mRNA was not observed in all macrophages in human atherosclerotic lesions (10). However, despite the importance of this receptor and the evidence for its regulation in vivo, very little is known about the factors that influence macrophage scavenger receptor regulation. In particular, in contrast to the LDL receptor, the scavenger receptor is not regulated by increased intracellular cholesterol content (5). Perhaps the most important aspect of its regulation studied to date in vitro has been the induction of scavenger receptor activity during the differentiation of monocytes into macrophages (13, 15). In this light, our finding that picomolar concentrations of TGF-β1 inhibit...
The mechanism of scavenger receptor regulation by TGF-β1 most directly involves a ~50% decrease in receptor mRNA (Fig. 5) which leads to a ~70% decrease in cell surface receptor number (Fig. 4). However, many of our degradation assays showed a ~70% inhibition by TGF-β1 (e.g. Figs. 2, 3, and 5). Thus, there may be an additional, albeit relatively minor, inhibitory effect of TGF-β1 that involves a post-binding step (e.g. internalization or lysosomal degradation). Scatchard analysis also revealed a decrease in receptor affinity (Fig. 4); however, all of our degradation experiments were conducted at saturating levels of [125I]-acetyl-LDL, and so this effect of TGF-β1 was probably not involved in the inhibition of [125I]-acetyl-LDL degradation observed in our experiments. The inhibition of ACAT activity (~80%; see Fig. 1) by TGF-β1 appeared to be even greater than that of [125I]-acetyl-LDL degradation. Rather than an independent ACAT-inhibitory effect of TGF-β1, however, this was probably due to the fact that when lipoprotein-cholesterol delivery to cells falls below a critical “threshold” level, ACAT activity is not stimulated (i.e. there is a nonlinear relationship between lipoprotein degradation and ACAT activity at low levels of lipoprotein degradation) (28).

In the absence of PMA-induced differentiation, scavenger receptor mRNA levels were not detectable by Northern blot analysis (data not shown; see above), whereas in the presence of PMA, scavenger receptor mRNA was markedly induced (Fig. 5 and Ref. 6). When PMA-induced differentiation was carried out in the presence of TGF-β1, scavenger receptor mRNA was decreased compared with that in cells differentiated in the absence of TGF-β1, but the mRNA was still detectable (Fig. 5). Thus, compared with undifferentiated THP-1 monocytes, scavenger receptor mRNA levels were only partially suppressed by TGF-β1. This finding, together with the finding that ~30% of scavenger receptor activity was not inhibited even at very high doses of TGF-β1 (Fig. 2), may indicate the presence of two classes of scavenger receptors: TGF-β1-sensitive and TGF-β1-resistant. Whether or not these two putative classes of scavenger receptors have different structures (e.g. type I versus type II receptors (Refs. 6 and 7)) or different functional attributes (e.g. differential abilities to bind acetyl-LDL versus oxidized LDL (Refs. 29 and 30) or lipoprotein ligands versus non-lipoprotein ligands (Refs. 12 and 31)) is yet to be determined.

What could be the physiological significance of our findings? In terms of atherosclerosis, the answer depends on whether macrophage foam cell formation is entirely a pathological event or, at least in the early stages of atherogenesis, a protective event (e.g. by scavenging harmful oxidized lipoproteins (8)). In the former case, TGF-β1-mediated inhibition of scavenger receptor activity (and thus foam cell formation) might be anti-atherogenic. Thus, one might postulate that a decrease in TGF-β1 secretion by arterial wall cells might accelerate the progression of atherosclerosis. In contrast, if foam cells are initially protective (8), then an increase in TGF-β1 secretion by arterial wall cells (18, 19) (which may occur in lesions (Ref. 32)) might contribute to the progression of atherosclerosis.

The macrophage scavenger receptor also binds LPS (12); since this occurs without a cytokine response, the function may be to scavenge or clear LPS from the circulation or tissues (12). Therefore, inhibition of scavenger receptor activity by TGF-β1 might be expected to delay LPS clearance and thus increase LPS effects, such as cytokine production by monocytes and macrophages. In this light, the finding by Wahl et al. (33) that TGF-β1 induces cytokine production in monocytes is consistent with an overall TGF-β1-mediated
increase in the inflammatory response of these cells.

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