

Regulation of Macrophage α_2 -Macroglobulin Receptor/Low Density Lipoprotein Receptor-related Protein by Lipopolysaccharide and Interferon- γ

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Abstract

α_2 -Macroglobulin receptor/low density lipoprotein receptor-related protein (α_2 M-R/LRP) is a broad specificity receptor that may function in lipoprotein metabolism, proteinase regulation, and growth factor regulation. In this study, we demonstrated that α_2 M-R/LRP expression in macrophages can be markedly decreased by LPS and by IFN- γ . Regulation of α_2 M-R/LRP in RAW 264.7 cells was demonstrated at the mRNA, antigen, and receptor-function levels. In receptor-function studies, the decrease in α_2 M-R/LRP expression was detected as a 90% decrease in the B_{max} or maximum receptor binding capacity for activated α_2 M after treatment with LPS or IFN- γ . Western blot analysis of whole cell lysates demonstrated significant loss of α_2 M-R/LRP heavy-chain. Northern blot analysis of poly(A)⁺ RNA revealed a marked decrease in α_2 M-R/LRP mRNA after treatment with LPS (79% decrease) or IFN- γ (70% decrease). Other cytokines, including tumor necrosis factor- α , transforming growth factor- β 1, and interleukin-6 did not regulate α_2 M-R/LRP. The ability of LPS and IFN- γ to regulate α_2 M-R/LRP was confirmed in experiments with primary cultures of murine bone marrow macrophages. These studies demonstrate that macrophage α_2 M-R/LRP is subject to significant downregulation by physiologically significant cytokines and signaling macromolecules. (*J. Clin. Invest.* 1993. 91:1219–1224.) Key words: proteinase inhibitor • cytokine • lipoprotein • apolipoprotein E • plasminogen activator inhibitor

Introduction

The α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein (α_2 M-R/LRP)¹ is a multifunctional recep-

tor expressed by a number of different cell types (1). α_2 M-R/LRP is synthesized as a 600-kD transmembrane glycoprotein and processed into 515- and 85-kD subunits in the *trans*-Golgi compartment (2, 3). The 85-kD subunit includes the transmembrane domain (3). The 515-kD subunit is bound to the 85-kD chain by noncovalent interactions and includes binding sites for a large number of ligands including: activated forms of the proteinase inhibitor, α_2 -macroglobulin (α_2 M*) (4–6); α_2 M*-growth factor complexes (7); pregnancy zone protein-proteinase complexes (8); apolipoprotein E-enriched lipoproteins (9); lipoprotein lipase (10); *Pseudomonas* exotoxin A (11); complexes of plasminogen activator inhibitor-I (PAI-1) with urokinase (uPA) or tissue-type plasminogen activator (12–14); and receptor-associated protein (RAP) (15–17). RAP is a 39-kD protein which inhibits binding of other ligands to α_2 M-R/LRP.

Due to the broad spectrum of ligands for α_2 M-R/LRP, it has been suggested that this receptor functions in numerous processes, including lipoprotein metabolism, hemostasis, cellular growth regulation, and tissue remodeling. The regulation of cellular α_2 M-R/LRP expression is not understood. Our laboratories recently demonstrated that the α_2 M* binding capacity of bone marrow macrophages is increased four- to fivefold by exposure to colony stimulating factor-1 (18). Other cytokines or cell signaling macromolecules that significantly affect α_2 M-R/LRP activity have not been identified. As proposed by Brown et al. (1), the identification of agents that downregulate α_2 M-R/LRP expression would provide a new approach for evaluating the function of this receptor in lipoprotein metabolism and other processes.

The activities of important macrophage receptors other than α_2 M-R/LRP are highly regulated. Bacterial LPS decreases surface-expression of scavenger receptors (19), mannose receptors (20), and tumor necrosis factor- α receptors (21). IFN- γ decreases expression of type I scavenger receptors (22) and transferrin receptors (23). TGF- β 1 decreases expression of types I and II scavenger receptors (24). Some of these regulatory activities may depend on the state of macrophage differentiation/activation.

The scavenger receptors (types I and II) are macrophage receptors implicated in atherogenesis (24, 25). Like α_2 M-R/LRP, scavenger receptors demonstrate broad ligand binding specificity. Neither receptor (scavenger receptor or α_2 M-R/LRP) is negatively regulated by cholesterol (1, 25). In this investigation, α_2 M-R/LRP regulation by cytokines and by LPS was studied in RAW 264.7 cells and bone marrow macrophages. The cytokines studied here have been shown to regulate scavenger receptor expression by other investigators. Our results demonstrate that IFN- γ and LPS significantly downreg-

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1. Abbreviations used in this paper: α_2 M*, activated α_2 -macroglobulin; α_2 M-R/LRP, α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein; B_{max} , maximum binding capacity; BMM, bone marrow macrophage; PGAD, phosphoglyceraldehyde dehydrogenase; RAP, receptor-associated protein; uPA, urokinase plasminogen activator.

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ulate $\alpha_2\text{M-R/LRP}$ function, as determined by $^{125}\text{I-}\alpha_2\text{M}^*$ binding. The decrease in function is associated with decreased $\alpha_2\text{M-R/LRP}$ antigen and mRNA levels. The pattern of $\alpha_2\text{M-R/LRP}$ regulation is similar to that previously reported for macrophage scavenger receptors.

Methods

Materials. Recombinant murine IFN- γ was supplied by Schering Co. (Kenilworth, NJ) or purchased from Genzyme Corp. (Cambridge, MA). The two preparations yielded equivalent results. Bacterial LPS (LPS W, *Escherichia coli* 0127:B8) was from Difco Laboratories Inc. (Detroit, MI). Recombinant murine tumor necrosis factor- α and recombinant murine interleukin-6 were supplied by R&D Systems (Minneapolis, MN). TGF- β 1 was purified from acid/ethanol extracts of human platelets as previously described (26). The sequences of human and murine TGF- β 1 are conserved with the exception of one amino acid (27). Each cytokine preparation contained < 0.5 ng/ml of endotoxin as determined by the limulus lysate assay.

$\alpha_2\text{M}$ was purified from human plasma by the method of Imber and Pizzo (28). The native form of $\alpha_2\text{M}$ demonstrates proteinase inhibitory activity but is not recognized by $\alpha_2\text{M-R/LRP}$ (29). $\alpha_2\text{M}$ that is reacted with proteinases or with small primary amines undergoes a major conformational change, exposing or orienting the receptor recognition site (one distinct site in each of the four $\alpha_2\text{M}$ subunits) (29). $\alpha_2\text{M}$ in the transformed, receptor-recognized conformation is termed activated $\alpha_2\text{M}$ ($\alpha_2\text{M}^*$). For the studies presented here, $\alpha_2\text{M}^*$ was generated by incubating $\alpha_2\text{M}$ with 200 mM methylamine HCl for 6 h at pH 8.2. $\alpha_2\text{M}^*$ was radiiodinated by the Iodobead method (Pierce Chemical Co., Rockford, IL) as described by the manufacturer. The specific activity was 0.3–0.8 $\mu\text{Ci}/\mu\text{g}$.

Cell culture. RAW 264.7 cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in T-75 flasks in RPMI 1640 (Sigma Immunochemicals, St. Louis, MO) with 10% FCS (Heartline; Sigma Immunochemicals) at 37°C, 5% CO_2 , and 95% humidity. Every 2–3 d, cells were detached by gentle scraping and passaged. For experiments, 4.0×10^5 RAW 264.7 cells were seeded per well in 24-well tissue culture plates (Costar Corp., Cambridge, MA) and grown to confluence (12–24 h).

Femoral and tibial bone marrow cells were isolated from BALB/C mice and cultured in DMEM (430–1600; Gibco Laboratories, Grand Island, NY) with 15% FCS, 1.0% penicillin-streptomycin, 50 μM mercaptoethanol, 0.02 mg/ml L-asparagine, and 5.0% TC-1-conditioned medium. TC-1 conditioned medium is a rich source of colony stimulating factor-1 (30). Essentially homogeneous preparations of bone marrow macrophages (BMM) were prepared from the marrow cell isolates as previously described (18). For experiments, the BMMs were plated at 3.0×10^5 cells/well in 24-well tissue culture plates and cultured for 48 h.

$\alpha_2\text{M}^*$ Binding studies. RAW 264.7 cells were incubated with LPS, IFN- γ , or other cytokines in serum-free RPMI 1640 for various periods of time. BMMs were incubated with the same agents in fully supplemented DMEM. After treatment, the RAW 264.7 cells and the BMM cultures were washed twice with Earle's balanced salts solution containing 10 mM Hepes, pH 7.4, and 1.0 mg/ml bovine serum albumin. Cellular binding of $^{125}\text{I-}\alpha_2\text{M}^*$ (0.1–5.0 nM) was studied at 4°C in the presence and absence of 0.2 μM nonradiolabeled $\alpha_2\text{M}^*$. Incubations were terminated after 4 h by separating the media from the cells. The wells were then washed three times and the cells lysed in 0.1 M NaOH, 1.0% SDS. Cell-associated radioactivity was determined in a model 1275 Minigamma gamma counter (LKB Instruments Inc., Gaithersburg, MD). Cellular protein concentration was determined by the method of Lowry et al. (31). Nonspecific binding was defined by the cell-associated radioactivity detected after incubation with 0.2 μM nonradiolabeled $\alpha_2\text{M}^*$. Specific binding was determined by subtracting nonspecific from total binding. Experiments were performed in quadruplicate unless otherwise specified. Each data point in a given

experiment represented a triplicate determination. Binding isotherms were fit by nonlinear regression to the equation for a rectangular hyperbola. The same data were also analyzed using the Scatchard transformation. The K_D and B_{max} (maximum binding capacity) were then determined by linear regression. The reported binding parameters were calculated by averaging the values determined for each independent study and are presented with the standard error of the mean (SEM). Alterations in $\alpha_2\text{M}^*$ binding to BMMs reflect the activities of the cytokines in the presence of 5.0% TC-1 conditioned medium.

Western blot analysis of $\alpha_2\text{M-R/LRP}$. RAW 264.7 cells were grown to confluence in T-75 flasks and then treated with IFN- γ (50 U/ml) or LPS (100 ng/ml) for 18 h. The cells were washed and then lysed in 2.0% SDS with 0.02 mg/ml leupeptin and 1.0 mM PMSF. Cell lysates were centrifuged at 10,000 g for 15 min. Protein concentrations were determined by the method of Lowry et al. (31). Equivalent amounts of cellular protein (0.2 mg) were subjected to SDS-PAGE as previously described (32). Western blot analysis was performed using polyclonal antibody R777 directed against human $\alpha_2\text{M-R/LRP}$. R777 was kindly provided by Dr. Dudley Strickland (American Red Cross, Rockville, MD). The gel slabs were soaked for 0.5 h in 20 mM Tris, 150 mM NaCl, pH 7.4. The proteins were then electrotransferred to Immobilon-P membranes (Millipore Corp., Bedford, MA) for 12 h at 0.4 A. The transfer buffer was 20 mM Tris-HCl, 150 mM glycine, 20% (vol/vol) methanol. The membranes were blocked with 5% nonfat milk for 1 h, washed, and incubated with R777 (1:1,000) for 1.5 h. Antibody binding was detected with goat anti-rabbit immunoglobulin peroxidase conjugate (1:1,000; Atlantic Antibody, Incstar Co., Stillwater, MN) followed by 3'-3'-diaminobenzidine-tetrahydrochloride (0.3 mg/ml) in 50 mM Tris-HCl, pH 7.4, and 0.01% H_2O_2 .

Northern blot analysis of $\alpha_2\text{M-R/LRP}$ mRNA. Poly(A)⁺ RNA was isolated from confluent RAW 264.7 cells using the Fast Track procedure (Invitrogen, San Diego, CA). RNA (4.0 μg) from control cells (untreated), cells treated with LPS (100 ng/ml), and cells treated with IFN- γ (50 U/ml) was separated on 0.8% agarose gels and electrotransferred to nylon membranes (Zeta probe; Bio-rad Laboratories, Richmond, CA). A cDNA probe specific for human $\alpha_2\text{M-R/LRP}$ mRNA was generously provided by Dr. Joachim Herz, Southwestern Medical Center, University of Texas. The probe was labeled with [α - ^{32}P]dCTP by nick translation and incubated with the nylon membranes containing transferred RNA at 42°C in 5 \times SSC, 5 \times Denhardt's reagent, 50 mM sodium phosphate, 50% formamide, 0.1% SDS, and 100 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA. Membranes were rinsed with 2 \times SSC and washed three times for 0.5 h in 2 \times SSC, 0.1% SDS at 68°C, and exposed to Kodak X-Omat AR-5 film at -70°C. As a control for load, membranes were hybridized with a [α - ^{32}P]dCTP-labeled cDNA probe for phosphoglyceraldehyde dehydrogenase (PGAD).

Results

$\alpha_2\text{M}^*$ Binding to RAW 264.7 cells. RAW 264.7 cells were maintained in serum-free medium for 18 h before performing $^{125}\text{I-}\alpha_2\text{M}^*$ binding experiments. $\alpha_2\text{M}^*$ binding was specific and saturable (Fig. 1 A). Nonspecific binding accounted for $< 35\%$ of total binding within the $\alpha_2\text{M}^*$ concentration range studied (up to 5.0 nM). The K_D and B_{max} were 0.6 ± 0.1 nM and 33.5 ± 1.2 fmol/mg cell protein ($n = 4$), respectively. When $\alpha_2\text{M}^*$ binding was studied without prior serum deprivation (single experiment), the K_D (0.8 nM) and B_{max} (37 fmol/mg cell protein) were approximately the same.

RAW 264.7 cells were treated with 100 ng/ml LPS for 18 h at 37°C in serum-free RPMI. The treated cells were then incubated with different concentrations of $^{125}\text{I-}\alpha_2\text{M}^*$ at 4°C in two separate experiments to generate complete binding isotherms. The results of one experiment are shown in Fig. 1 A. The K_D was unchanged (0.6 nM) while the B_{max} was decreased by $> 90\%$ (2.3 fmol/mg cell protein). In the second experiment,

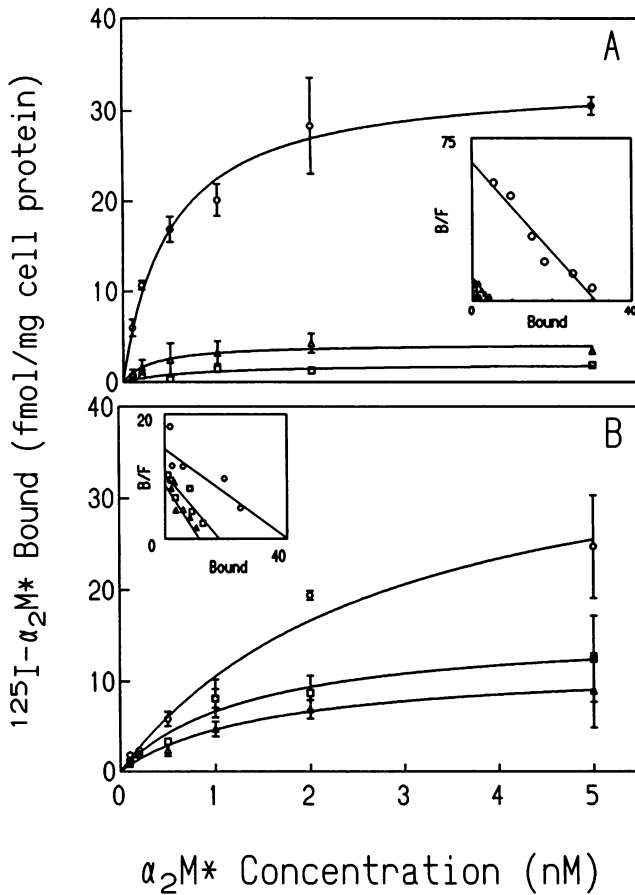


Figure 1. Specific binding of $^{125}\text{I}-\alpha_2\text{M}^*$ to RAW 264.7 cells and BMMs before and after treatment with LPS or IFN- γ . In *A*, binding isotherms and Scatchard transformations (*inset*) are shown for the binding of $^{125}\text{I}-\alpha_2\text{M}^*$ to RAW 264.7 cells (\circ), and to RAW 264.7 cells treated with 100 ng/ml LPS (\square) or 50 U/ml IFN- γ (Δ). In *B*, binding isotherms and Scatchard transformations (*inset*) are shown for the binding of $^{125}\text{I}-\alpha_2\text{M}^*$ to BMMs (\circ), and to BMMs treated with 200 ng/ml LPS (\square) or 50 U/ml IFN- γ (Δ). Binding studies were performed after continuously exposing RAW 264.7 cells or BMMs to LPS or IFN- γ for 18 h.

specific binding of $^{125}\text{I}-\alpha_2\text{M}^*$ was completely eliminated (within the sensitivity limits of the technique).

Specific binding of $\alpha_2\text{M}^*$ to RAW 264.7 cells was also significantly decreased when the cells were treated with IFN- γ (50 U/ml) for 18 h (Fig. 1 *A*). The K_D remained unchanged (0.4 nM) while the B_{max} was decreased to 4.6 fmol/mg cell protein (86% decrease, average of two experiments). RAW 264.7 cells demonstrated unchanged $\alpha_2\text{M}^*$ specific binding activity when incubated for up to 24 h with any of the following individually: TGF- β 1 (1.0 ng/ml), interleukin-6 (2.0 ng/ml), or tumor necrosis factor- α (10 or 100 U/ml).

In control experiments, $^{125}\text{I}-\alpha_2\text{M}^*$ (5.0 nM) and LPS (1.0 $\mu\text{g}/\text{ml}$) were incubated together with RAW 264.7 cells at 4°C. No change in specific $\alpha_2\text{M}^*$ binding was observed (data not shown). Therefore, LPS does not directly compete for $\alpha_2\text{M}^*$ binding sites.

$\alpha_2\text{M}^*$ Binding to bone marrow macrophages. Cell-surface expression of $\alpha_2\text{M}-\text{R}/\text{LRP}$ in murine BMMs has been demonstrated previously (18). Survival and proliferation of these cells and expression of $\alpha_2\text{M}-\text{R}/\text{LRP}$ requires colony stimulating

factor-1. Therefore, BMMs were treated with LPS (200 ng/ml) or IFN- γ (50 U/ml) in the presence of TC-1 conditioned medium (a source of colony stimulating factor-1) for 18 h. $\alpha_2\text{M}^*$ binding was then studied (Fig. 1 *B*). The K_D and B_{max} for untreated cells were 2.3 ± 0.2 nM and 43 ± 6 fmol/mg cell protein, respectively ($n = 4$). In LPS-treated cells, the B_{max} was decreased to 20 ± 5 fmol/mg cell protein and the K_D was unchanged (2.1 ± 0.2 nM). IFN- γ treatment caused a 70% decrease in B_{max} (13 ± 2 fmol/mg cell protein); the K_D was 1.6 ± 0.2 nM. The response of BMMs to LPS and IFN- γ was somewhat less than that observed with RAW 264.7 cells. The difference may reflect the more complex BMM culture medium.

Time and concentration dependency of $\alpha_2\text{M}-\text{R}/\text{LRP}$ modulation in RAW 264.7 cells. RAW 264.7 cells treated with 100 ng/ml LPS or 50 U/ml IFN- γ demonstrated minimal change in $\alpha_2\text{M}^*$ binding for up to 10 h (Fig. 2). The decrease in binding observed with either agent by 18 h was relatively stable since no further decrease was apparent at 24 h.

The decrease in $\alpha_2\text{M}^*$ binding to RAW 264.7 cells was LPS and IFN- γ concentration-dependent. Significantly decreased $\alpha_2\text{M}^*$ binding ($P < 0.05$) was observed after treating cells with LPS at concentrations as low as 1.0 ng/ml. The lowest concentration of IFN- γ that caused a statistically significant decrease in $\alpha_2\text{M}^*$ binding was 2.5 U/ml (data not shown).

RAW 264.7 cells were pulse exposed to LPS (100 ng/ml) or IFN- γ (50 U/ml) for different time periods (Fig. 3). $\alpha_2\text{M}^*$ binding was then examined 18 h after pulse treatment was initiated. Cells exposed to LPS for 3 h or more demonstrated the maximum decrease in $\alpha_2\text{M}^*$ binding capacity. Cells exposed to IFN- γ for 1 h or more were maximally affected.

Western blot analysis of $\alpha_2\text{M}-\text{R}/\text{LRP}$ in RAW 264.7 cells. Whole cell SDS-extracts of RAW 264.7 cells were subjected to Western blotting using antibody R777. This antibody was raised against human $\alpha_2\text{M}-\text{R}/\text{LRP}$ but cross-reacts with the

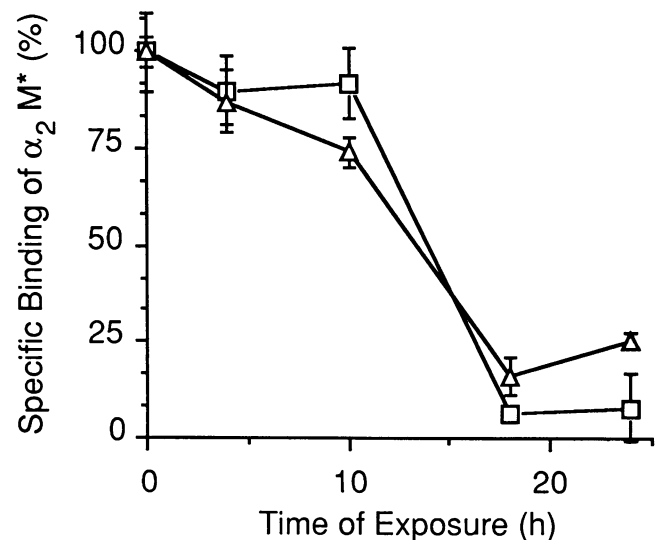


Figure 2. $\alpha_2\text{M}^*$ binding to RAW 264.7 cells treated with LPS or IFN- γ for different time periods. Confluent RAW 264.7 cells were exposed for different lengths of time to 50 U/ml IFN- γ (Δ) or 100 ng/ml LPS (\square). Immediately after exposure, binding of 2.0 nM $^{125}\text{I}-\alpha_2\text{M}^*$ (with and without 0.2 μM nonradiolabeled $\alpha_2\text{M}^*$) was studied. Specific binding is expressed as a percentage (mean \pm SEM) of that demonstrated by cells incubated in serum-free medium for 24 h.

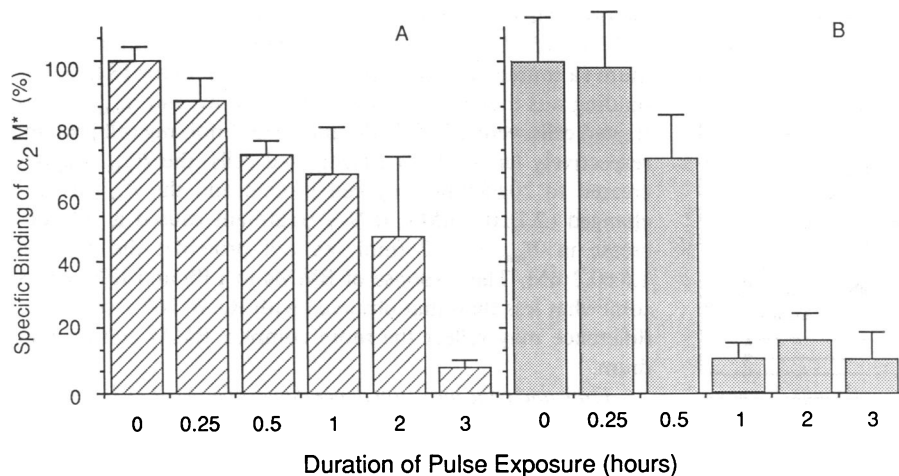


Figure 3. α_2M^* binding to RAW 264.7 cells pulse exposed to LPS or IFN- γ . RAW 264.7 cells were pulse exposed to 100 ng/ml LPS or 50 U/ml IFN- γ for different amounts of time. The cells were then washed and incubated in medium without LPS or IFN- γ . α_2M^* binding (2.0 nM ^{125}I - α_2M^* with or without 0.2 μ M nonradiolabeled α_2M^*) was studied 18 h after initiating exposure to LPS (A) or IFN- γ (B). Specific binding is expressed as a percentage of that demonstrated by untreated cells.

murine α_2M -R/LRP heavy chain (11). A single band with the expected mass of the α_2M -R/LRP heavy chain was detected (Fig. 4). The 85-kD chain and RAP were not detected (possibly due to a sensitivity problem in this cross-species system). The intensity of the 500-kD band was proportional to the amount of cellular extract loaded in the gel (data not shown). Treatment of the RAW 264.7 cells with IFN- γ (50 U/ml) or LPS (100 ng/ml) significantly decreased α_2M -R/LRP-heavy chain antigen expression in four separate experiments.

Northern blot analysis of α_2M -R/LRP mRNA in RAW 264.7 cells. To determine whether the decrease in α_2M -R/LRP function and antigen were mediated at the mRNA level, Northern blot analysis was performed on poly(A)⁺ RNA extracts of RAW 264.7 cells. A single 15.0-kb RNA hybridized with the cDNA probe, as expected for α_2M -R/LRP (Fig. 5). The level of α_2M -R/LRP mRNA was decreased in cells treated with LPS or IFN- γ . By densitometric scanning, the mRNA levels were decreased by 79% with LPS and by 70% with IFN- γ (assuming equal load based on PGAD hybridization).

Discussion

In this investigation, we demonstrated markedly decreased specific binding of α_2M^* to macrophages treated with IFN- γ or

LPS. The decrease in specific binding was due to a change in the number of receptors per cell (B_{max}) and not receptor affinity. The mechanism of receptor downmodulation was evaluated in Western and Northern blotting experiments. After treatment with LPS or IFN- γ , α_2M -R/LRP antigen and mRNA were decreased. Therefore, α_2M -R/LRP expression in macrophages is subject to downregulation by naturally occurring signaling molecules.

The studies presented here and elsewhere (19, 22) suggest that α_2M -R/LRP and macrophage scavenger receptors are downregulated similarly by LPS and IFN- γ . LDL receptor expression is apparently not regulated by either agent (19, 22). A difference in the pattern of regulation of α_2M -R/LRP and scavenger receptors may have been revealed by our studies with TGF- β 1. This cytokine did not affect α_2M -R/LRP expression in RAW 264.7 cells. By contrast, Bottalico et al. (24) demonstrated significant downregulation of scavenger receptors by TGF- β 1 in phorbol-ester treated THP-1 cells. While these two receptors may be regulated differently in macrophages by TGF- β 1, it is also possible that the apparent difference reflects the cell-type studied (THP-1 versus RAW 264.7 cells) or the state of cellular differentiation/activation.

The loss of α_2M -R/LRP (determined by α_2M^* binding) in response to LPS or IFN- γ can probably be explained entirely by the decrease in cellular antigen and mRNA. The role of RAP as a regulator of α_2M -R/LRP function in LPS or IFN- γ -

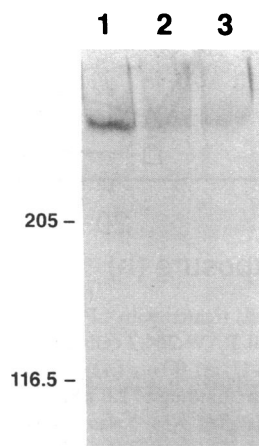


Figure 4. Western blot analysis of α_2M -R/LRP in RAW 264.7 cells exposed to LPS or IFN- γ . RAW 264.7 cells were treated with IFN- γ (50 U/ml) or LPS (100 ng/ml) for 18 h. The cells were then lysed in 2.0% SDS with leupeptin and PMSF. Identical amounts of cellular protein (0.2 mg) were subjected to SDS-PAGE. After electrotransfer to Immobilon-P membranes (Millipore Corp.), α_2M -R/LRP was detected with polyclonal antibody R777. Lane 1, untreated RAW 264.7 cells; lane 2, LPS-treated cells; lane 3, IFN- γ -treated cells. Molecular mass markers are shown on the side.

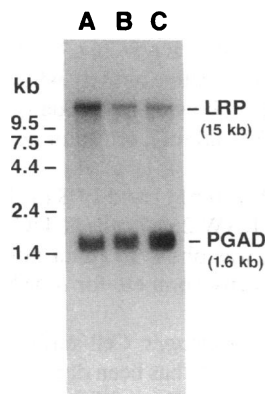


Figure 5. Northern blot analysis of α_2M -R/LRP mRNA. Poly(A)⁺ RNA was isolated from untreated RAW 264.7 cells (A), cells treated with 100 ng/ml LPS for 18 h (B), and cells treated with 50 U/ml IFN- γ for 18 h (C). After electrophoresis and electrotransfer to nylon membranes, the blot was first probed for α_2M -R/LRP mRNA (revealing only the 15-kb RNA) and then for PGAD.

treated cells was not directly evaluated. By binding to α_2 M-R/LRP, RAP competitively decreases binding of other ligands including α_2 M* (15, 16). If the level of RAP remained unchanged while α_2 M-R/LRP levels decreased, then the increase in the ratio of RAP to α_2 M-R/LRP may have contributed to the decrease in α_2 M* binding capacity.

While numerous functions for α_2 M-R/LRP have been suggested based on the ligands that are recognized, many questions regarding how α_2 M-R/LRP affects cell and organ function remain unanswered. We previously identified α_2 M-R/LRP as a receptor for cytokines which are bound to α_2 M* (7, 33). Unpublished studies from our laboratory suggest that the effect of α_2 M on TGF- β 1 activity in vitro depends on cellular expression of α_2 M-R/LRP. Such studies imply that cells may alter responsiveness to cytokines by regulating α_2 M-R/LRP.

Recognition of uPA-PAI-1 complexes (not free uPA) represents another important function of α_2 M-R/LRP (12–14). uPA, when bound to uPAR (the cellular uPA receptor), initiates an enzyme cascade which promotes cellular migration (34). uPAR-associated uPA reacts rapidly with PAI-1 and the resulting inactive complex may remain uPAR-associated (35–37) unless it is cleared by α_2 M-R/LRP. Therefore, α_2 M-R/LRP may promote cellular migration by stripping uPAR of uPA-PAI-1 complex, so that the receptor is available for active uPA. Other investigators have demonstrated that IFN- γ increases the level of cellular fibrinolytic activity by increasing uPAR expression and/or uPA secretion (38, 39). The regulation of α_2 M-R/LRP by IFN- γ suggests that this cytokine regulates multiple factors functioning in proteinase-mediated cellular migration. By decreasing plasma membrane levels of α_2 M-R/LRP, IFN- γ may decrease the efficiency of the uPA-initiated proteinase cascade, thereby regulating the impact of increased cellular uPA or uPAR expression.

Finally, regulation of α_2 M-R/LRP might also affect cellular uptake of lipoproteins and exotoxins. The functional consequences of α_2 M-R/LRP in lipid metabolism may be difficult to assess due to the concurrent downmodulation of scavenger receptors (19, 22); however, decreased uptake of apo-E-containing lipoproteins may influence atherogenesis (40).

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References

1. Brown, M. S., J. Herz, R. C. Kowal, and J. L. Goldstein. 1991. The low density lipoprotein receptor-related protein: double agent or decoy? *Curr. Opin. Lipidol.* 2:65–72.
2. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl, and K. K. Stanley. 1988. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:4119–4127.
3. Herz, J., R. C. Kowal, J. L. Goldstein, and M. S. Brown. 1990. Proteolytic processing of the 600 kd low density lipoprotein receptor-related protein (LRP) occurs in the trans-golgi compartment. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1769–1776.
4. Strickland, D. K., J. D. Ashcom, S. Williams, W. H. Burgess, M. Migliorini,

and W. S. Argraves. 1990. Sequence identity between the α_2 -macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this is a multifunctional receptor. *J. Biol. Chem.* 265:17401–17404.

5. Moestrup, S. K., and J. Gliemann. 1989. Purification of the rat hepatic α_2 -macroglobulin receptor as an approximately 440-kDa single chain protein. *J. Biol. Chem.* 264:15574–15577.

6. Moestrup, S. K., and J. Gliemann. 1991. Analysis of ligand recognition by the purified α_2 -macroglobulin receptor (low density lipoprotein receptor-related protein). *J. Biol. Chem.* 266:14011–14017.

7. LaMarre, J., M. A. Hayes, G. K. Wollenberg, I. Hussaini, S. W. Hall, and S. L. Gonias. 1991. An α_2 -macroglobulin receptor-dependent mechanism for the plasma clearance of transforming growth factor- β 1 in mice. *J. Clin. Invest.* 87:39–44.

8. Moestrup, S. K., E. I. Christensen, L. Sottrup-Jensen, and J. Gliemann. 1987. Binding and receptor-mediated endocytosis of pregnancy zone protein-proteinase complex in rat macrophages. *Biochim. Biophys. Acta.* 930:297–303.

9. Beisiegel, U., W. Weber, G. Ihrke, J. Herz, and K. K. Stanley. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature (Lond.)* 341:162–164.

10. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* 88:8342–8346.

11. Kounnas, M. Z., R. E. Morris, M. R. Thompson, D. J. FitzGerald, D. K. Strickland, and C. B. Saelinger. 1992. The α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein binds and internalizes *Pseudomonas* exotoxin A. *J. Biol. Chem.* 267:12420–12423.

12. Nykjaer, A., C. M. Petersen, B. Moller, P. H. Jensen, S. K. Moestrup, T. L. Holtet, M. Etzerodt, H. C. Thogersen, M. Munch, P. A. Andreasen, and J. Gliemann. 1992. Purified α_2 -macroglobulin receptor/LDL receptor-related protein binds urokinase-plasminogen activator inhibitor type-1 complex. *J. Biol. Chem.* 267:14543–14546.

13. Orth, K., E. L. Madison, M.-J. Gething, J. F. Sambrook, and J. Herz. 1992. Complexes of tissue-type plasminogen activator and its serpin inhibitor plasminogen activator inhibitor type 1 are internalized by means of the low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor. *J. Biol. Chem.* 89:7422–7426.

14. Bu, G., S. Williams, D. K. Strickland, and A. L. Schwartz. 1992. Low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. *Proc. Natl. Acad. Sci. USA.* 89:7427–7431.

15. Williams, S. E., J. D. Ashcom, W. S. Argraves, and D. K. Strickland. 1992. A novel mechanism for controlling the activity of α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein. *J. Biol. Chem.* 267:9035–9040.

16. Herz, J., J. L. Goldstein, D. K. Strickland, Y. K. Ho, and M. S. Brown. 1991. 39-kDa protein modulates binding of ligands to low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor. *J. Biol. Chem.* 266:21232–21238.

17. Strickland, D. K., J. D. Ashcom, S. Williams, F. Battey, E. Behre, K. McTigue, J. F. Battey, and W. S. Argraves. 1991. Primary structure of α_2 -macroglobulin receptor-related protein. *J. Biol. Chem.* 266:13364–13369.

18. Hussaini, I. M., K. Srikumar, P. J. Quesenberry, and S. L. Gonias. 1990. Colony stimulating factor-1 modulates α_2 -macroglobulin receptor expression in murine bone marrow macrophages. *J. Biol. Chem.* 265:19441–19446.

19. Van Lenten, B. J., A. M. Fogelman, J. Seager, E. Ribbi, M. D. Haberlson, and P. A. Edwards. 1985. Bacterial endotoxin selectively prevents the expression of scavenger-receptor activity on human monocyte-macrophages. *J. Immunol.* 134:3718–3721.

20. Shepherd, V. L., R. Abdolrasulnia, M. Garrett, and H. B. Cowan. 1990. Down-regulation of mannose receptor activity in macrophages after treatment with lipopolysaccharide and phorbol esters. *J. Immunol.* 145:1530–1536.

21. Ding, A. H., E. Sanchez, S. Srimal, and C. F. Nathan. 1989. Macrophages rapidly internalize their tumor necrosis factor receptors in response to bacterial lipopolysaccharide. *J. Biol. Chem.* 264:5129–5135.

22. Geng, Y., and G. K. Hansson. 1992. Interferon- γ inhibits scavenger receptor expression and foam cell formation in human monocyte-derived macrophages. *J. Clin. Invest.* 89:1322–1330.

23. Hamilton, T. A., J. E. Weiel, and D. O. Adams. 1984. Expression of the transferrin receptor in murine peritoneal macrophages is modulated in the different stages of activation. *J. Immunol.* 132:2285–2290.

24. Bottalico, L. A., R. E. Wager, L. B. Agellon, R. K. Assoian, and I. Tabas. 1991. Transforming growth factor- β 1 inhibits scavenger receptor activity in THP-1 human macrophages. *J. Biol. Chem.* 266:22866–22871.

25. Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witzum. 1989. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* 320:615–624.

26. Assoian, R. K. 1987. Purification of type- β transforming growth factor from human platelets. In *Methods Enzymol.* Barnes, D., and D. A. Sirbasku, editors. Vol. 146. Academic Press, Inc., Orlando, FL. pp. 153–163.

27. Derynck, R., J. A. Jarrett, E. Y. Chen, and D. V. Goeddel. 1986. The murine transforming growth factor- β precursor. *J. Biol. Chem.* 261:4377-4379.
28. Imber, M. J., and S. V. Pizzo. 1981. Clearance and binding of two electrophoretic fast forms of α_2 -macroglobulin. *J. Biol. Chem.* 256:8134-8139.
29. Sottrup-Jensen, L. 1987. α_2 -macroglobulin and related thiol ester proteases. In *The Plasma Proteins*. F. W. Putnam, editor. Academic Press, Inc., Orlando, FL. 192-291.
30. Song, Z. X., R. K. Shaddock, D. J. Innes, A. Waheed, and P. J. Quesenberry. 1985. Hematopoietic factor production by a cell line (TC-1) derived from adherent murine marrow cells. *Blood.* 66:273-281.
31. Lowry, O. H., N. S. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
32. Gonias, S. L., and N. L. Figler. 1988. α_2 -Macroglobulin is the primary inhibitor of miniplasmin in vitro and in vivo in the mouse. *Biochem. J.* 255:725-730.
33. Gonias, S. L. 1992. α_2 -Macroglobulin: a protein at the interface of hemostasis and cellular growth regulation. *Exp. Hematol. (NY)*. 20:302-311.
34. Ellis, V., and K. Dano. 1992. The urokinase receptor and the regulation of cell surface plasminogen activation. *Fibrinolysis.* 6 (Suppl. 4):27-34.
35. Ellis, V., T. C. Wun, N. Behrendt, E. Ronne, and K. Dano. 1990. Inhibition of receptor-bound urokinase by plasminogen-activator inhibitors. *J. Biol. Chem.* 265:9904-9908.
36. Estreicher, A., J. Muhlhauser, J.-L. Carpentier, L. Orci, and J.-D. Vassalli. 1990. The receptor for urokinase type plasminogen activator polarizes expression of the protease to the leading edge of migrating monocytes and promotes degradation of enzyme-inhibitor complexes. *J. Cell Biol.* 111:783-792.
37. Cubellis, M. V., T.-C. Wun, and F. Blasi. 1990. Receptor-mediated internalization and degradation of urokinase is caused by a specific inhibitor PAI-1. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:1079-1085.
38. Lu, H., P. Mirshahi, P. Krief, C. Soria, J. Soria, Z. Mishal, O. Bertrand, J. Y. Perrot, H. Li, C. Picot, et al. 1988. Parallel induction of fibrinolysis and receptors for plasminogen and urokinase by interferon gamma on U937 cells. *Biochem. Biophys. Res. Commun.* 155:418-422.
39. Mirshahi, S. S., J. Soria, H. Lu, E. Pujade-Lauraine, M. Mirshahi, and C. Soria. 1992. Effect of interferon gamma and adriamycin on fibrinolytic activity associated to human monocytes. *Fibrinolysis.* 6(Suppl. 4):157-160.
40. Brown, M. S., and J. L. Goldstein. 1992. Koch's postulates for cholesterol. *Cell.* 71:187-188.