Metabolism and Turnover of Cell Surface–Associated Heparan Sulfate Proteoglycan and Chondroitin Sulfate Proteoglycan in Normal and Cholesterol-Enriched Macrophages

Rick T. Owens and William D. Wagner

Analysis of sulfur-35-labeled proteoglycans indicated that cholesterol-enriched pigeon peritoneal macrophages synthesized 42% more 35S-labeled proteoglycan when compared with control macrophages during a 24-hour incubation. Proteoglycan turnover was subsequently studied in radiolabeled macrophage cultures after a 1-, 3-, 6-, 12-, or 24-hour chase with fresh media. During the chase, intracellular proteoglycan disappeared rapidly, whereas there was a small accumulation of 35S-labeled proteoglycan in the media that plateaued at about 6 hours and remained relatively constant thereafter. Pericellular heparan sulfate proteoglycan and chondroitin sulfate proteoglycan disappeared throughout the chase and did not appear to accumulate in the media or in the intracellular compartment. The rapid disappearance of intracellular proteoglycans along with the relative lack in metabolism of media proteoglycans indicated that the majority of pericellular proteoglycans were metabolized via an intracellular degradative pathway. Kinetic analysis of pericellular proteoglycans revealed the presence of a single pool of heparan sulfate proteoglycan (half-life [t1/2] = 6.9 hours) and a single pool of chondroitin sulfate proteoglycan (t1/2 = 11.5 hours) in control macrophage cultures. Cholesterol-enriched macrophage cultures also contained a single pool of pericellular heparan sulfate proteoglycan (t1/2 = 7.3 hours) but contained two pools of chondroitin sulfate proteoglycan (t1/2 = 0.8 hour and 25.9 hours).

Arteriosclerosis and Thrombosis 1991;11:1752-1758

Proteoglycans (PGs) are a group of macromolecules characterized by the covalent association of one or more glycosaminoglycan (GAG) chains to a core protein. Heterogeneity in core protein structure as well as GAG number and composition is responsible for the structural diversity of PGs, which in turn imparts a broad range of functional activities. Extracellular matrix PGs function primarily in the regulation of tissue permeability and the maintenance of the structural integrity of tissues through interactions with other matrix components such as hyaluronic acid, fibronectin, and collagen. Similar interactions involving cell surface PGs suggest that these molecules may function as matrix anchors or receptors. Several additional receptors, including the fibroblast transferrin receptor and transforming growth factor-β receptor type III, also have been identified as PGs. Furthermore, the major histocompatibility complex class II-associated invariant chain found on the surface of antigen-presenting cells has been identified as a PG and may be involved in the immune response. Additional evidence for the role of PGs in immunity is based on the presence of PGs in the intracellular secretory granules of mast cells, polymorphonuclear leukocytes, and natural killer cells. These PGs serve to bind and package granule proteases, thereby preventing self-lysis.

Lymphocytes and macrophages also produce PGs, but unlike other immune cells, the majority are secreted and not stored intracellularly. Recently, we have examined the production of PGs by cultured pigeon peritoneal macrophages (Owens and Wagner, unpublished observations). These cells synthesized several distinct PGs that were either secreted or...
expressed in pericellular or intracellular compartments. Secreted PGs consisted of a large-size 6-sulfated chondroitin sulfate PG (CS-PG), a smaller 4-sulfated CS-PG, and a heparan sulfate PG (HS-PG). Trypsin treatment of the cell layer released a 6-sulfated CS-PG and an HS-PG, indicating a close association to the cell surface for these two PGs. Analysis of the remaining intracellular compartment revealed the absence of intact PGs and the presence of CS GAG and HS GAG chains, consistent with degradative processing of PGs.

To understand the metabolism and interrelation of the secreted, cell surface, and intracellular PGs, a pulse-chase study was designed. PGs were isolated from cultures of control and cholesterol-enriched macrophages obtained from the peritoneal cavities of normocholesterolemic and hypercholesterolemic pigeons. Cholesterol-enriched macrophages were used to model foam cells typical of those found within atherosclerotic lesions and were examined to determine if cholesterol loading induced changes in PG turnover or metabolism that might be related to lesion progression. Cholesterol enrichment of macrophages has varied effects on function, including enhanced fibrinolytic activity, decreased production of hydrogen peroxide and arachidonic acid metabolites, increased release of tumor necrosis factor, increased elastase activity, and decreased interleukin-1 production, as well as stimulation of apolipoprotein E gene expression. The degree of stimulation or inhibition of macrophage function in the previously mentioned studies was in some cases dependent on the procedure used to cholesterol-load the macrophages. In this study, macrophages were loaded with cholesterol in vivo in an attempt to mimic processes that may occur during atherosclerosis.

Methods

Materials

Sulfur-35-labeled sodium sulfate was purchased from Amersham Corp. (Arlington Heights, Ill.). Tissue-culture dishes were obtained from Corning, (Corning, N.Y.), and all other tissue-culture reagents were from Flow Labs, Inc. (Rockville, Md.). Benzmimidine hydrochloride, 6-aminohexanoic acid, butyl nitrite, and 1-hexadecylpyridinium chloride (CPC) were purchased from Eastman Kodak Co. (Rochester, N.Y.). CPC was recrystallized twice from acetone/water (1:2) before use. Tryptamine hydrochloride and phenanthroline were from Sigma Chemical Co. (St. Louis, Mo.), chondroitin ABC lyase (Proteus vulgaris) was from Miles Laboratories (Elkhart, Ind.), chondroitin sulfate was from Nutritional Biomedicals Corp. (Cleveland, Ohio), and Diaflo membranes were from Amicon (Danvers, Mass.). The cholesterol assay kit was purchased from Boehringer Mannheim Diagnostics (Indianapolis, Ind.). All other chemicals used were obtained from Fisher Scientific Co. (Pittsburgh, Pa.). White Carneau pigeons were obtained from a closed colony maintained at the Pigeon Research Center of Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. Pigeons made hypercholesterolemic were fed a diet consisting of Purina pigeon pellets containing 0.5% cholesterol and 10% lard (wt/wt) for 5 weeks before necropsy.

Cell Culture

Thioglycollate-elicited macrophages were isolated from the peritoneal cavity of normocholesterolemic and hypercholesterolemic pigeons as previously described. Briefly, macrophages were elicited by an intraperitoneal injection of 5 ml sterile 3% thioglycollate broth. Two days later, peritoneal cells were obtained by washing the peritoneal cavity with phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, 8.1 mM Na2HPO4, [pH 7.4]) containing 10 units/ml heparin. Cells were then plated in 100-mm culture dishes containing 10 ml Eagle's minimal essential medium supplemented with Eagle's minimal essential medium vitamins, 200 mM l-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum. After 2 hours at 37°C in 95% air and 5% CO2, nonadherent cells were removed by thoroughly washing the cell layer three times with 5 ml PBS, and fresh media were added. After a 20-hour incubation, adherent cells were washed once with PBS and incubated with fresh media containing 50 µCi/ml [35S]sodium sulfate.

Initially, macrophages were isolated from three normocholesterolemic pigeons and three hypercholesterolemic pigeons. Cells from individual pigeons were cultured separately and radiolabeled for 24 hours in the presence of 50 µCi/ml [35S]sodium sulfate. Radiolabeled PGs were subsequently isolated from the macrophage cultures as described below. The pulse-chase experiment was performed with a single pool of cells obtained from either eight normocholesterolemic pigeons or eight hypercholesterolemic pigeons. After a 24-hour incubation in the presence of 50 µCi/ml [35S]sodium sulfate, radiolabeling media were removed, and the radiolabeled cell layer was washed three times with 5 ml PBS. Radiolabeled PGs were then chased with fresh media containing 1 mM Na2SO4. Triplicate cultures were examined at 0, 1, 3, 6, 12, or 24 hours.

Proteoglycan Isolation

Radiolabeled macrophage cultures were divided into media, pericellular, and intracellular compartments. Culture media were removed, the cell layer was washed twice with 2.5 ml PBS, and the washes were added to the media. Culture media were then centrifuged at 800g for 10 minutes to remove cell debris. The cell layer was treated with 0.6 ml 0.05% trypsin and 0.02% EDTA for 5 minutes at 37°C. Culture dishes were then rinsed with PBS, and the cells were pelleted at 450g for 10 minutes. The trypsin supernatant (pericellular compartment) was removed, and the remaining cell pellet (intracellular compartment) was washed and resuspended in 1 ml 30 mM Na2SO4 and 20 mM NaCl. Cell suspensions

Downloaded from http://atvb.ahajournals.org/ by guest on April 1, 2017
were sonicated, and aliquots were removed for cell cholesterol determination (Boehringer Mannheim Single Vial cholesterol reagent) and total cell protein determination by the method of Lowry et al.\textsuperscript{22}

Protease inhibitors (100 mM 6-aminohexanoic acid, 10 mM Na\textsubscript{2}EDTA, 5 mM benzamidine hydrochloride, 2.5 mM phenanthroline, and 5 mM tryptamine hydrochloride) were added to the culture media and pericellular fluids before extensive dialysis against 30 mM Na\textsubscript{2}SO\textsubscript{4} and 20 mM NaCl containing protease inhibitors. One hundred microliters of carrier chondroitin sulfate was added to the dialyzed media, the pericellular samples, and the intracellular samples. PGs were then precipitated with 1% CPC for 24 hours at 25°C. PG–CPC complexes were pelleted at 800g for 2 hours, and the complexes were dissociated by the addition of 1 ml 2 M NaCl/ethanol (100:14). PGs free of CPC were precipitated by the addition of 2 ml absolute ethanol. Precipitated PGs were then washed with a series of graded ethanol washes (90%, 95%, and 100%) before being dried.

**Proteoglycan Analysis**

PGs were resuspended in 1 ml 0.05 M sodium acetate, pH 5.8, and aliquots were removed for determination of \textsuperscript{35}S radioactivity. Triplicate samples were pooled, and PGs were precipitated in the presence of 70% ethanol for 18 hours at \(-20^\circ C\). Precipitated PGs were resuspended in 100 mM Tris(hydroxymethyl)aminomethane (Tris), 30 mM sodium acetate, and 10 mM EDTA (pH 8.0) and identified based on susceptibility to degradation with chondroitin ABC lyase or nitrous acid. Enzyme digestions were performed with 0.01 unit chondroitin ABC lyase in the presence of 0.036 mM pepstatin, 0.5 mM phenylmethylsulfonyl fluoride, and 10 mM \(\text{NaClO}_3\). PG degradation was determined by the appearance of low-molecular-weight \textsuperscript{35}S-labeled material after chromatography on a 7.5x300-mm TSK 5000 PW column with 0.25 M Tris phosphate, pH 7.6, at a flow rate of 0.5 ml/min.

**Kinetic Analysis**

Experimental equations describing the disappearance of cell surface PGs were generated by the Simulation Analysis and Modeling (SAAM) program.\textsuperscript{25} This program is designed to generate exponential equations that best describe curvilinear data. Best-fit equations (\(p<0.05\)) were determined by comparing residual sums of squares obtained from monoexponential and biexponential equations by Fisher’s \(F\) test.\textsuperscript{26}

**Results**

Macrophages isolated from the peritoneal cavity of hypercholesterolemic pigeons contained significantly more cholesterol (124±24.0 \(\mu\)g/mg cell protein) compared with macrophages isolated from the peritoneal cavity of normocholesterolemic pigeons (28±3.1 \(\mu\)g/mg cell protein). After a 24-hour incubation in the presence of \([\text{35}S]\)sodium sulfate, the overall distribution of \textsuperscript{35}S-labeled PGs into media (49–66%), pericellular (17–31%), and intracellular (11–24%) compartments was similar in cultures of control and cholesterol-enriched macrophages. However, cholesterol-enriched macrophages produced significantly more radiolabeled PG compared with control macrophages (Figure 1). This was reflected by increased levels of \textsuperscript{35}S-labeled PGs in the culture media as well as in the pericellular and intracellular compartments.

Radiolabeled macrophage cultures were chased with fresh media for 1, 3, 6, 12, or 24 hours, and \textsuperscript{35}S-labeled PGs were isolated by precipitation with CPC (Figure 1). In both control and cholesterol-enriched macrophage cultures, intracellular \textsuperscript{35}S-labeled PGs disappeared rapidly during the chase. Pericellular PGs disappeared throughout the chase but appeared to do so at different rates in cultures of control and cholesterol-enriched macrophages. Low levels of radiolabeled PGs appeared in the media and plateaued at about 6 hours. In both types of macrophage cultures, only 13% of the radiolabeled PGs present in the intracellular and pericellular compartments at time zero was recovered from the media after 24 hours. The relative lack of accumulation of radiolabeled PGs within the media or within the intracellular compartment suggested that the fate of the majority of pericellular PGs, once removed from the cell surface, was degradation. The absence of a precursor–product relation between pericellular and media PGs also suggested that the majority of PGs secreted into the culture medium (comprising about 60% of the \textsuperscript{35}S-labeled PGs produced after 24 hours of labeling) arose primarily from newly synthesized molecules and not from existing pericellular or intracellular pools.

The apparent difference in turnover of cell surface PGs between control and cholesterol-enriched macrophages was examined further. Pericellular PGs were composed entirely of HS-PGs and CS-PGs, as determined by complete susceptibility to nitrous acid.
Owens and Wagner  Cell Surface Proteoglycans in Macrophages 1755

FIGURE 1. Pulse-chase kinetics curves of proteoglycans (PGs) from control (panel A) and cholesterol-enriched (panel B) macrophage cultures. Control and cholesterol-enriched macrophage cultures were radiolabeled with 50 μCi/ml sulfur-35 sodium sulfate for 24 hours and chased for 0, 1, 3, 6, 12, or 24 hours with media containing 1 mM NaSO₄. PGs were isolated from media (○), pericellular (△), and intracellular (□) compartments by precipitation with 1-hexadecylpyridinium chloride. Values represent the mean±SEM of triplicate cultures.

(HS-PG) and chondroitinase ABC (CS-PG) treatment (data not shown). Relative amounts of HS and CS present in the pericellular compartment during the chase were determined based on susceptibility to nitrous acid treatment. After chromatography on a TSK 5000 column, degraded 35S-labeled material eluting at the column v₀ (HS-PG) and intact 35S-labeled material (CS-PG) were quantified by totaling the radioactivities represented by the respective peaks. At time zero, control cells contained 4.1 dpm/10⁶ cells of 35S-labeled CS-PGs and 3.0 dpm/10⁶ cells of 35S-labeled HS-PGs, whereas cholesterol-enriched cells contained 4.9 dpm/10⁶ cells of 35S-labeled CS-PGs and 6.5 dpm/10⁶ cells of 35S-labeled HS-PGs. Increased total pericellular PGs in cholesterol-enriched macrophages (Table 1) were therefore mainly due to increased levels of HS-PGs.

In both control and cholesterol-enriched macrophage cultures, the proportion of CS to HS increased steadily throughout the chase. This suggested that turnover of HS-PGs was more rapid compared with that of CS-PGs. Logarithmic plots of die-away curves obtained for pericellular HS-PGs were linear in cultures of control and cholesterol-enriched macrophages, suggesting a single pool of pericellular HS-PGs in both cell types (Figure 2). The die-away curve for pericellular CS-PGs was also linear in control macrophage cultures but was nonlinear in cholesterol-enriched macrophage cultures, suggesting more than one pool of pericellular CS-PGs in these cells. Kinetic analyses of die-away curves indicated the presence of a single pool of HS-PGs, with similar half-lives (t₁/₂) in cultures of control (t₁/₂ = 6.9±0.6 hours) and cholesterol-enriched (t₁/₂ = 7.3±0.2 hours) macrophages. Turnover rates for CS-PGs were significantly different in cultures of control and cholesterol-enriched macrophages and were also different compared with those of HS-PGs. Control macrophages contained one pool of CS-PG (t₁/₂ = 11.5±1.7 hours), whereas cholesterol-enriched macrophages had a fast turnover pool (t₁/₂ = 0.8±0.5 hour) as well as a slow turnover pool (t₁/₂ = 25.9±7.7 hours).

FIGURE 2. Disappearance curves of heparan sulfate proteoglycan (HS-PG) and chondroitin sulfate proteoglycan (CS-PG) from the macrophage cell surface. Control (panel A) and cholesterol-enriched (panel B) macrophage cultures were radiolabeled with 50 μCi/ml sulfur-35 sodium sulfate for 24 hours. After 0, 1, 3, 6, 12, or 24-hour chase with fresh media, pericellular (trypsin-releasable) PGs were isolated. Identification of PG was based on susceptibility to chondroitin ABC lyase or nitrous acid. Pericellular CS-PG (△) and HS-PG (○) are shown.
Radiolabeled PGs present in the media after the 24-hour chase were identified based on susceptibility to nitrous acid and chondroitinase ABC treatment. In both control and cholesterol-enriched macrophages, about 55% of the radiolabeled PG was HS-PG, based on response to degradation by nitrous acid. The remainder of the PGs were degraded by chondroitinase ABC, as demonstrated by complete degradation of 35SI-radioactivity after sequential nitrous acid and chondroitinase ABC treatment. PGs released into the media during the 24-hour chase therefore consisted of about 55% HS-PG and 45% CS-PG. PGs released into the media during the initial 24-hour labeling period contained 15% HS-PGs in control cultures and 11% HS-PGs in cholesterol-enriched macrophage cultures. The increased proportion of HS-PGs found in the media after the 24-hour chase suggested that some pericellular HS-PGs were released into the media, thereby increasing the relative amount of media HS-PGs. Although the data in Figure 1 did not demonstrate any relation between the disappearance of radiolabeled pericellular PGs and the appearance of radiolabeled PGs in the media, the release of only a small percentage of pericellular HS-PGs into the media (which, after a 24-hour chase, contained only 13% of the radiolabeled PG present in the pericellular and intracellular compartments at the beginning of the chase) would be sufficient to cause the increased proportion of HS-PGs observed in the media after the 24-hour chase.

Discussion

Hypercholesterolemic pigeons provided a source of cholesterol-enriched macrophages that contained significantly more cholesterol than did control macrophages obtained from normocholesterolemic pigeons. In vitro methods of cholesterol loading were not used for several reasons. In vitro methods involve the receptor-mediated uptake of modified lipoproteins, such as acetylated low density lipoprotein (LDL),

oxidized LDL, or dextran sulfate–conjugated LDL, and have not been shown to occur in vivo. Ligand binding to some of these receptors may stimulate macrophage function without inducing cholesterol accumulation. The exact mechanism of macrophage cholesterol accumulation in vivo is not known; therefore, isolation of macrophages from hypercholesterolemic animals provided a physiological source of cholesterol-enriched macrophages. Elicitation with thioglycollate was necessary to provide a sufficient number of macrophages.

Pulse-chase kinetics were used to assess the production and metabolism of PGs in cultures of control and cholesterol-enriched macrophages. Interrelations between secreted, cell surface, and intracellular PGs were demonstrated to be similar in both types of cultures. However, kinetic analysis revealed differences in turnover of cell surface PGs in control macrophages compared with cholesterol-enriched macrophages.

Although cultures of cholesterol-enriched macrophages synthesized significantly more 35SI-labeled PG compared with control cultures, the overall distribution into media, pericellular, and intracellular compartments was similar. After a chase with fresh media, intracellular 35SI-labeled material disappeared rapidly, whereas there was only minor accumulation of 35SI-labeled PGs in the media. The amount of media PGs plateaued at about 6 hours and remained constant throughout the remainder of the chase. Media PGs therefore appeared to turn over slowly and were not rapidly metabolized by either cell-mediated or extracellular mechanisms. Disappearance of 35SI-labeled pericellular PGs occurred throughout the 24-hour chase and was not associated with significant increases in intracellular or media pools of 35SI-labeled PGs. The majority of media PGs were therefore most probably derived from newly synthesized molecules and not from existing pericellular or intracellular pools of PGs. Increased proportions of HS-PGs observed in culture media after a 24-hour chase compared with that in the media used to radiolabel the cells was, however, suggestive of a small amount of pericellular HS-PGs being released into the culture media. The remaining pericellular PGs did not accumulate in the media or intracellular compartment and were therefore most likely degraded. The relative lack in metabolism of media PGs and the rapid disappearance of intracellular 35SI-labeled material suggested that pericellular PGs were internalized and degraded intracellularly. Previous studies in pigeon macrophages (Owens and Wagner, unpublished observations) as well as the M132 and U-93733 macrophage-like cell lines have demonstrated the presence of intracellular GAGs and GAG fragments, supporting the concept of intracellular degradative processing of PGs by macrophages.

Internalization and degradation of pericellular PGs have been described in various cell types, including rat ovarian granulosa cells, human colon carcinoma cells, bovine smooth muscle cells, and a rat hepatocyte cell line. In rat ovarian granulosa cells, PGs were degraded by several different pathways. Sixty percent of internalized HS-PGs and dermatan sulfate (DS) PGs underwent rapid degradation (t1/2=0.5 hour) without producing detectable intermediates. The remaining DS-PG was only partially degraded, yielding intact GAG chains (t1/2=4 hours), whereas the remaining HS-PG was first degraded to GAG fragments that were one third their original size (t1/2=0.5 hour), followed by additional endoglycosidic cleavage, producing GAG chains one fourth to one fifth their original size (t1/2=0.5–1 hour) before complete degradation (t1/2=3–4 hours). One or more of these pathways may exist in pigeon macrophages, providing a mechanism for the uptake and degradation of pericellular PGs.

Pericellular PGs are also released into the culture media by a variety of cell types. Mechanisms of
release are dependent on the mode of association of the PGs with the cell membrane. Ionically associated PGs can be displaced by other molecules. Alternatively, membrane-associated molecules that bind PGs may be shed or cleaved, thereby releasing the PGs into the culture media. Integral membrane PGs can also be shed into the media or proteolytically cleaved in a manner similar to that described for cell surface HS-PGs found on bovine smooth muscle cells. However, as mentioned earlier, only a minor proportion of pericellular PGs were released into the culture media by pigeon macrophages.

Pericellular HS-PG was lost from the cell surface at similar rates ($t_{1/2}$=7 hours) in cultures of control and cholesterol-enriched pigeon macrophages. There was, however, an increased amount of $^{35}$S-labeled HS-PGs on the surface of cholesterol-enriched cells. These cell surface HS-PGs could be important in mediating cellular adhesion through interactions with other matrix components such as collagen and fibronectin. Guinea pig peritoneal macrophages expressed a particular type of cell surface HS when grown in monolayer compared with suspension cultures, supporting a role for HS-PGs in macrophage adhesion. Furthermore, increased cell surface HS-PG may be responsible for the increased adhesion observed for cholesterol-enriched rat peritoneal macrophages to bovine aortic endothelial cells and vascular smooth muscle cells. In these studies, Rogers et al demonstrated a 50–80% increase in the number of cholesterol-enriched macrophages compared with control cells adherent to monolayers of endothelial or smooth muscle cells.

Rates of loss of pericellular CS-PGs were different in cultures of control and cholesterol-enriched macrophages as well as being different from that of HS-PG. Control macrophages contained a single pool of pericellular CS-PG ($t_{1/2}$=11.5 hours), whereas cholesterol-enriched cells contained two pools ($t_{1/2}$=0.8 hour and $t_{1/2}$=25.9 hours). One possible explanation for this difference centers on the role of macrophages as antigen-presenting cells. The major histocompatibility complex class II-associated invariant chain, previously described as a CS-PG, has been reported to be rapidly internalized and degraded after cell surface expression on splenocytes. Rapid internalization of CS-PG may therefore be suggestive of enhanced major histocompatibility complex class II antigen expression or antigen-presenting capacities by cholesterol-enriched macrophages.

There is reason to believe that macrophage cell surface CS-PGs also are involved in cell adhesion. Kolset et al has demonstrated that CS-PGs produced by human macrophages and mouse macrophages bind to collagen–Sepharose and fibronectin–Sepharose affinity columns. Differences in pericellular CS-PG metabolism may also therefore be related to the increased adhesive properties of cholesterol-enriched rat macrophages as mentioned above. The greater synthetic capacity of cholesterol-enriched macrophages led to an increase in the amount of $^{35}$S-labeled PG released into the media in cultures of cholesterol-enriched macrophages. These secreted PGs can become incorporated as part of the extracellular matrix or function in an as-yet-undefined capacity. Some potential roles of secreted PGs would include acting as carrier molecules for one or more of the many macrophage secretory products in a manner similar to the interaction described between platelet factor 4 and the small CS-PG released by platelets. Secreted PGs may also be involved in mediating the cytolytic activity of macrophages, as is thought to be the case for natural killer cells. In natural killer cells, release of PG was directly correlated with the ability of cells to lyse susceptible tumor cell targets. Although many of the proposed functions of macrophage PGs are purely speculative, it is obvious that these molecules have the potential to mediate a wide array of macrophage activities.

Acknowledgments

The authors wish to thank Dawn C. Schwenke for performing the kinetic analysis on the disappearance of cell surface PG; D.L. Cutter, I.J. Edwards, J.S. Parks, T.C. Register, D.C. Schwenke, and R.W. St. Clair for their critical reviews of the manuscript; and Lonnie Ellis for assistance in preparation of the manuscript.

References

13. Stevens RL: Secretory granule proteoglycans of mast cells and natural killer cells, in Functions of the Proteoglycans. Chich-
KEY WORDS: macrophages • heparan sulfate • chondroitin sulfate • proteoglycans • cholesterol


25. Oike Y, Kimata K, Shinomura T, Nakazawa K, Suzuki S: Structural analysis of chick-embryo cartilage proteoglycan by selective degradation with chondroitin lyases (chondroitinases) and endo-


27. Montgomery RR, Cohn ZA: Endocytic and secretory reper-

28. Parthsarathy S, Fong LG, Citero D, Steinberg D: Recognition of solubi


40. Rogers KA, Hoover RL, Castellot JJ Jr, Robinson JM, Kar-


43. Parthsarathy S, Fong LG, Citero D, Steinberg D: Recognition of solubilized apoproteins from delipidated, oxidized low density lipoprotein (LDL) by the acetyl-LDL receptor. Proc Natl Acad Sci U S A 1987;84:537-540
Metabolism and turnover of cell surface-associated heparan sulfate proteoglycan and chondroitin sulfate proteoglycan in normal and cholesterol-enriched macrophages.

R T Owens and W D Wagner

_Arterioscler Thromb Vasc Biol._ 1991;11:1752-1758
doi: 10.1161/01.ATV.11.6.1752

_Arteriosclerosis, Thrombosis, and Vascular Biology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/11/6/1752

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Arteriosclerosis, Thrombosis, and Vascular Biology_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Arteriosclerosis, Thrombosis, and Vascular Biology_ is online at:
http://atvb.ahajournals.org/subscriptions/