DEGRADATION OF CONNECTIVE TISSUE MATRICES BY MACROPHAGES

II. Influence of Matrix Composition on Proteolysis of Glycoproteins, Elastin, and Collagen by Macrophages in Culture*

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The breakdown of connective tissue macromolecules in both normal and pathological states is thought to require the active participation of stimulated macrophages. These phagocytic cells produce degradative enzymes, including plasminogen activator (1), elastase (2), and collagenase (3), that are likely mediators for macrophage-induced hydrolysis of connective tissue proteins (4). Because stimulated mouse peritoneal macrophages are easily obtained and cultured outside the animal, their neutral and lysosomal proteinases have been characterized with the use of purified substrates (2, 3, 5). However, these experiments are remote from the normal and pathological digestion of mixtures of proteins in vivo (4, 6). This is an important consideration because the connective tissue components may interact in such a way as to modify their accessibility to, and degradation by, specific hydrolytic enzymes.

In a previous report (4), we have shown that the matrix of insoluble extracellular proteins produced by rat vascular smooth muscle cells (SMC)[1] in culture can serve as a substrate for cells with degradative potential, and that proteinases produced by macrophages degrade the glycoprotein, elastin, and collagen of the SMC matrix. In the present work we examined, in detail, the degradation of the glycoprotein, elastin, and collagen components of connective tissue by live stimulated macrophages and attempted to define the interrelationships and relative contributions of the individual macrophage enzymes to the degradation of the extracellular matrix.

Materials and Methods

Except when described below, all methods and materials used are described in the previous report (4).

Phase-Contrast Microscopy. Macrophages were cultured on matrix in 24-well plates as described (4) except that cells were plated at $5 \times 10^4/cm^2$. Cells were fixed with 1.25% glutaraldehyde in phosphate-buffered saline, the bottom 2 mm of the wells was cut off, and

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[1] *Abbreviations used in this paper:* Plg, plasminogen; SMC, smooth muscle cell(s); TG, thioglycollate elicited.

cells and matrix were examined and photographed with a Zeiss Photomicroscope III equipped with a 40 × phase-contrast water-immersion lens (Carl Zeiss, Inc., New York).

Results

Degradation of Extracellular Matrix of SMC. Thioglycollate-elicited (TG) macrophages, plated in acid-treated fetal bovine serum, were active in the solubilization of radioactivity from [^{3}H]proline-labeled SMC extracellular matrix (Fig. 1). Degradation of matrix was dependent both on the time of incubation and on the number of macrophages plated. The degradative activity of as few as 3.3×10^4 TG macrophages was detected reproducibly. Radioactivity was solubilized initially in the form of large peptides, which were subsequently degraded to oligopeptides and amino acids (4, 7, 8).

Role of Plasminogen in Degradation of Matrix. The role of plasminogen (Plg) in degradation of SMC matrix was investigated because Plg stimulates the degradation of glycoproteins, which are plasmin-sensitive (4), because it stimulates degradation of collagen by activating latent forms of collagenase (9), and because it stimulates degradation by living macrophages (7). TG macrophages were plated in serum-free medium in the presence or absence of human Plg on matrices prelabeled with [^{3}H]-fucose, which selectively labeled matrix glycoproteins, or [^{3}H]proline, which labeled all matrix proteins. There was little solubilization of radioactivity (<2%) from either of the labeled matrices in the absence of Plg. Plg stimulated the solubilization of 17% of the radioactivity from the [^{3}H]proline-labeled matrix and was even more effective in stimulating that of the [^{3}H]fucose-labeled matrix (44%). These data suggest that macrophages cultured on the matrix in the presence of Plg have an accelerated rate of glycoprotein hydrolysis.

Rates of Solubilization of Glycoproteins, Elastin, and Collagen from SMC Matrices. In the insoluble extracellular matrix glycoproteins are the components most accessible to macrophages (8). It was therefore important to establish the fate of the elastin and collagen components of the matrix when the glycoproteins have been degraded by macrophages.

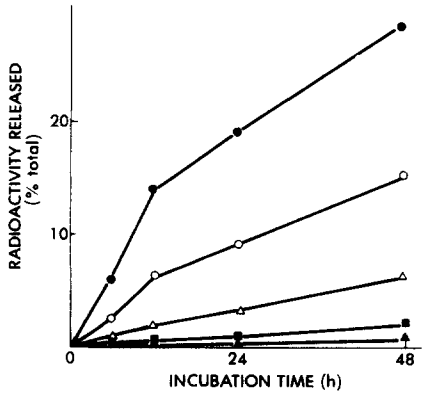

FIG. 1. Kinetics of solubilization of radioactivity from [^{3}H]proline-labeled R22 SMC matrices by TG macrophages. TG macrophages were plated in the presence of 10% acid-treated fetal bovine serum on the complete [^{3}H]proline-labeled extracellular matrix produced by R22 SMC. The solubilization of radioactivity from cultures plated with 10^4 (▲), 3.3×10^4 (■), 10^5 (△), 3.3×10^5 (○), or 10^6 (●) macrophages was then determined at the times indicated.

Plasmin or trypsin was used to deplete the extracellular matrix of glycoproteins and the subsequent rates of digestion of the residual matrix and of its elastin component were investigated (Table I). Pretreatment with plasmin, and particularly with trypsin, markedly accelerated the rate of digestion of the total matrix by macrophages. Digestion of elastin was increased 3- and 10-fold after plasmin and trypsin pretreatment, respectively. The results could not be explained by residual trypsin or plasmin acting as activators of latent forms of macrophage proteinases because addition of soybean trypsin inhibitor (60 μg/ml) or α_1-proteinase inhibitor (100 μg/ml) to the cultures had no effect on the rate of elastin digestion.

The mechanism by which pretreatment with trypsin accelerated the rate of matrix degradation was investigated further in an experiment in which varying amounts of the matrix glycoproteins were removed before addition of macrophages (Fig. 2). The rate of total matrix digestion and the amount of elastin digested by the macrophages were clearly dependent on the extent of glycoprotein-depletion by pretreatment with

TABLE I

Effect of Enzyme Pretreatment on Degradation of R22 SMC Matrix by Macrophages

Enzyme pretreatment	Total radioactivity solubilized by macrophages	Elastin degraded by macrophages (percent of total)
	cpm × 10^{-3}	
None	6.2	3.2
Plasmin, 10 μg/ml	9.7	9.5
Trypsin, 10 μg/ml	33.6	31.5

TG macrophages (5×10^5) were plated on the complete R22 SMC matrix or on matrices pretreated with trypsin or plasmin for 24 h. The matrices had been biosynthetically labeled with [^{3}H]proline, and the total radioactivity solubilized by the macrophages was determined after 48 h in the absence of Plg. Elastin degradation was determined by sequential enzyme digestion (4).

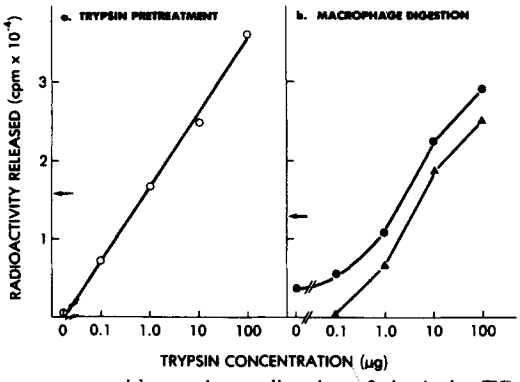

FIG. 2. Effect of pretreatment with trypsin on digestion of elastin by TG macrophages. SMC matrices labeled with [^{3}H]proline were treated with the indicated concentrations of trypsin for 5 h at 37°C, and the amount of radioactivity solubilized (a) was determined. Macrophages were then cultured on these matrices in the absence of Plg (b), and the total radioactivity (●) and the amount of elastin (▲) solubilized in 48 h were determined. The arrows indicate (a) the extent of hydrolysis of matrix by five sequential digestions with 10 μg/ml plasmin and (b) the amount of elastin digested from this matrix by macrophages in 48 h.

trypsin. Exhaustive plasmin treatment released only 40% of the glycoproteins, and the plasmin-treated matrix was subsequently degraded by the macrophages to the same extent as a matrix pretreated with a trypsin concentration of equivalent effectiveness (1 μg/ml). Although trypsin and plasmin may nick elastin without solubilizing peptides, the susceptibility of purified bovine ligament elastin to degradation by live macrophages and by macrophage elastinolytic enzymes was not increased after enzyme pretreatment (data not shown). These results indicate that degradation of elastin by macrophages is markedly stimulated if the glycoprotein components of the matrix are first removed.

Our other experiments have shown that purified macrophage elastase is less active in the hydrolysis of glycoproteins from the matrix than is plasmin (4). The substrate specificity of live macrophages was therefore examined in more detail (Table II). After incubation with macrophages, the matrices were analyzed by sequential treatment with trypsin, porcine pancreatic elastase, and clostridiopeptidase A for the amount of each component digested. The complete matrix was digested slowly, as found in other experiments in the absence of Plg. Addition of Plg increased the extent of glycoprotein digestion considerably, suggesting again that plasmin was responsible for the hydrolysis of glycoproteins. Small quantities of elastin and collagen (2–9%) were also digested. However, 32% of the elastin was digested by the macrophages from trypsin-pretreated matrix in the presence or absence of Plg, showing that Plg had no direct effect on elastin digestion in the absence of matrix glycoproteins. Collagen digestion was also detected when macrophages were cultured on trypsin-pretreated matrices, suggesting that glycoprotein removal also stimulated the degradation of collagen.

Phase-contrast microscopy of macrophages cultured on complete or glycoprotein-depleted matrices in the absence or presence of Plg confirmed the biochemical findings (Fig. 3). There was an accelerated rate of dissolution of identifiable elastin particles when macrophages were cultured on glycoprotein-depleted matrices. Although mac-

TABLE II

Comparison of the Rates of Degradation of Glycoproteins, Elastin, and Collagen from Complete and Glycoprotein-depleted SMC Matrix by Macrophages

Matrix pretreatment	Plg added	Matrix component digested		
		Glycoproteins	Elastin	Collagens
	μg/ml		%	
None	0	7.6 ± 5.0	6.3 ± 4.3	1.8 ± 1.5
	10	60.6 ± 11.8	9.2 ± 6.6	3.3 ± 2.7
Trypsin, 10 μg/ml	0	NP*	32.1 ± 1.7	8.6 ± 9.1
	10	NP	29.8 ± 7.6	4.6 ± 5.5

TG macrophages (5×10^5) were plated on complete or glycoprotein-depleted matrices in the presence or absence of human Plg. After 48 h, the macrophages were removed by NH_4OH lysis and the composition of the residual matrix was analyzed by sequential enzyme digestion (4). The percentage of each matrix component that had been digested by the macrophages was then calculated by subtraction of these values from those obtained for controls incubated without cells. Results are expressed as the mean ± SD for duplicate samples from three separate experiments.

* NP, none present.

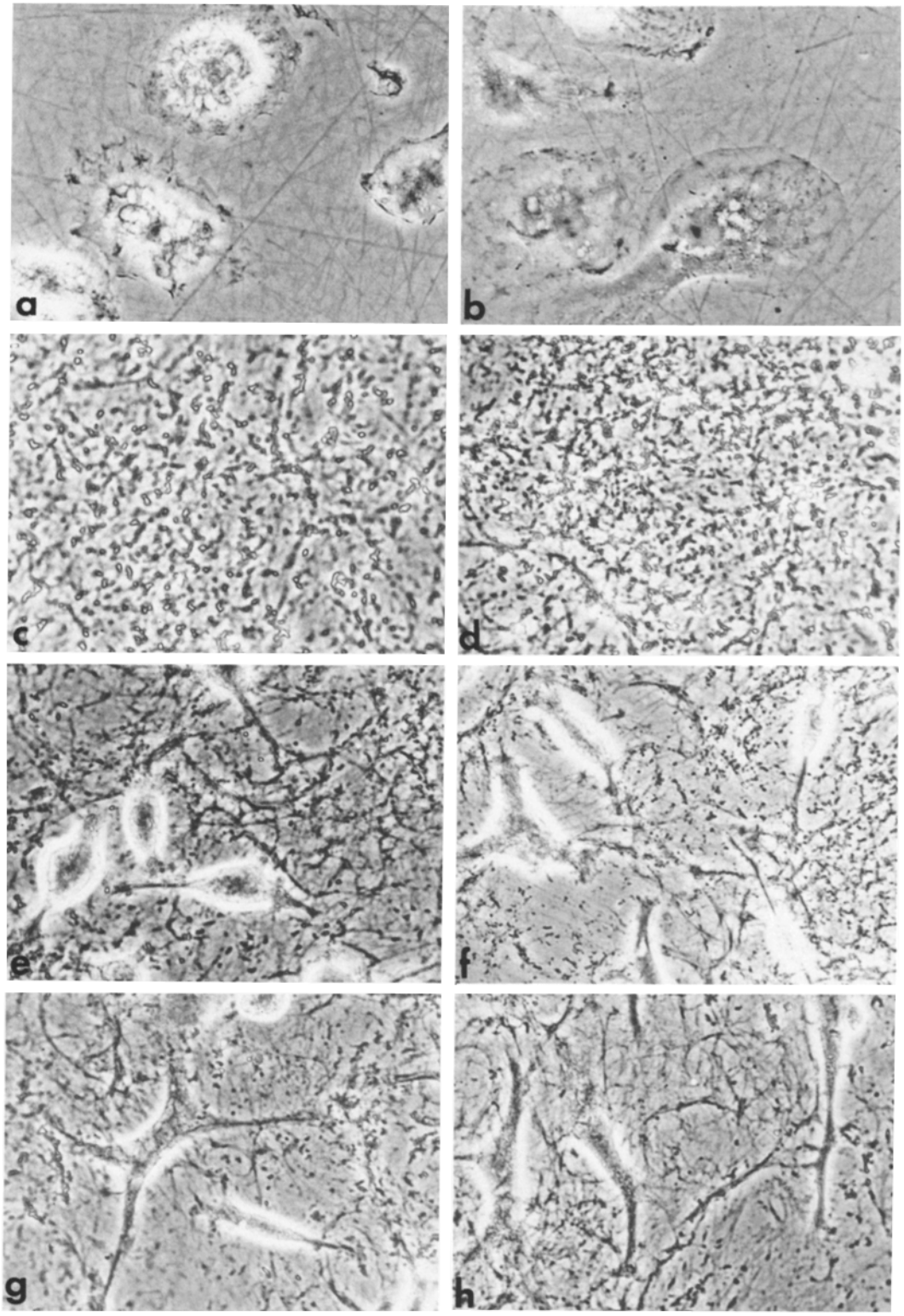

FIG. 3. Morphology of macrophages and matrix after 72 h of culture. Macrophages cultured in plastic culture wells in (a) medium without Plg, or (b) medium that contained 10 μg Plg/ml. Cell-free SMC matrix that is (c) complete, or (d) depleted of glycoproteins by pretreatment with trypsin. Macrophages cultured on complete matrix in (e) medium without Plg, or (f) medium with Plg. Macrophages cultured on matrix pretreated with trypsin in (g) medium without Plg, or (h) medium with Plg. Note that small refractile particles of the matrix correspond to elastin and the fine fibrillar material corresponds to collagen. No morphologically identifiable structure corresponds to glycoproteins. Phase-contrast: × 500.

rophages did not adhere as well to the matrix as to plastic culture wells, there was increased spreading in the presence of Plg and as the digestion of matrix progressed.

Rates of Degradation of Complete and Glycoprotein-depleted Matrix by Live Macrophages and Macrophage-conditioned Medium. If all the degradation of matrix by live macrophages were mediated by secreted proteinases, then the degradation of matrix by the cells or their conditioned medium would be the same when adjusted for equivalent proteolysis. We also assumed that secretion of proteinases was linear with time. Medium conditioned by TG macrophages was as active in hydrolysis of matrix as live cells, both in the absence and presence of Plg (Table III). However, conditioned medium hydrolyzed matrix to a greater extent than live cells under several of the experimental conditions described in Table III, suggesting that labile inhibitors might be closely associated with the phagocytes. Such inhibitors of elastase are secreted by macrophages (4). Differences in matrix degradation could also occur if secretion of proteinases was not linear with time. Colchicine, which increased the secretion of both elastase and Plg activator (10), markedly stimulated the rate of digestion of complete and glycoprotein-depleted matrices by both macrophages and conditioned medium.

Requirements for RNA and Protein Synthesis during Degradation of Extracellular Matrix by Macrophages. The rate of degradation of glycoprotein-depleted matrices in the absence of Plg was inhibited 91% by cycloheximide and 70% by actinomycin D, inhibitors of RNA and protein synthesis (Table IV). The production of elastase was therefore very sensitive to RNA and protein synthesis inhibition. The rate of hydrolysis of the complete matrix in the presence of Plg was inhibited only 40% by cycloheximide and 17% by actinomycin D. Previous studies have shown that considerable quantities of Plg activator are associated with macrophage lysates (1), but that little elastase can be demonstrated intracellularly (2). The expression of the catalytic activity of Plg

TABLE III

Comparison of Matrix Degradation by Cultured TG Macrophages and Macrophage-conditioned Medium

Macrophage treatment	Plg added	Enzyme secreted in medium		Radioactivity solubilized (percent of total) from complete matrix by		Radioactivity solubilized (percent of total) from glycoprotein-depleted matrix by	
		Elastase	Plg activator	Live macrophages	Conditioned medium	Live macrophages	Conditioned medium
	μg/ml	*U/ml*	*U/ml*				
None	0	0.7	5.4	1.1	1.2	10.7	11.4
	10	1.1	ND*	23.8	34.2	25.1	10.6
Colchicine, 2 μM	0	3.1	12.2	4.0	5.2	31.5	62.4
	10	4.3	ND	40.3	51.9	56.7	75.0

TG macrophages (1×10^6) were plated in plastic wells or on [^{3}H]proline-labeled complete or glycoprotein-depleted R22 SMC matrices in the absence or presence of bovine Plg for 48 h. Cells were treated with 2 μM colchicine as indicated to increase proteinase secretion. The conditioned medium from the cells grown in plastic wells was placed on matrices for 24 h (equivalent to growing cells on matrix for 48 h). Radioactivity solubilized by live cells at 48 h or by conditioned medium at 24 h was measured. Aliquots of medium from a parallel set of wells were then assayed for macrophage elastase activity on elastin in the absence of sodium dodecyl sulfate (4, 5) or for Plg activator on ^{125}I-fibrin in the presence of 0.15 M NaCl. The distribution of total radioactivity in the complete matrices was 38%, 54%, and 8% and in glycoprotein-depleted matrices 3%, 81%, and 16% glycoprotein, elastin, and collagen, respectively.

* ND, not determined.

TABLE IV

Effects of Inhibitors of RNA and Protein Synthesis on Digestion of R22 SMC Matrix by Macrophages

Inhibitor added	Plg added	Radioactivity solubilized from matrix	
		Complete	Glycoprotein-depleted
	μg/ml	*cpm × 10⁻³*	
None	0	0.8	10.3
Cycloheximide	0	0.4	0.9
Actinomycin D	0	0.4	3.1
None	10	21.4	6.4
Cycloheximide	10	12.9	0.9
Actinomycin D	10	17.6	3.0

TG macrophages (1×10^6) were plated on complete or glycoprotein-depleted R22 SMC matrices, which had been prelabeled with [^{3}H]proline, in the presence or absence of human Plg. The cells were washed after 3 h and the medium was replaced with medium that contained 1 μg/ml cycloheximide or 0.1 μg/ml actinomycin D. The total radioactivity solubilized was determined after 24 h.

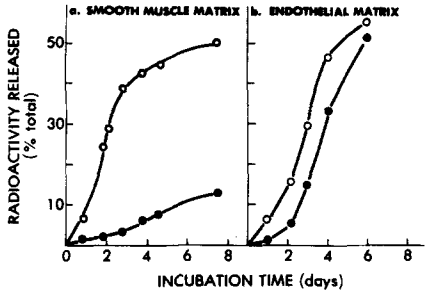

FIG. 4. Comparison of the degradation of (a) smooth muscle cell and (b) endothelial cell extracellular matrices by macrophages. TG macrophages (5×10^5) were incubated on matrix in serum-free medium in the absence (●) or presence (○) of 10 μg/ml of bovine Plg. Degradation of connective tissue components in the presence of Plg was determined by sequential enzyme digestion (4). Percentage of the components digested was (a) glycoprotein, 67%, elastin, 56%, and collagen, 8%; and (b) glycoprotein, 72%, and collagen, 21%.

activator is therefore less sensitive to RNA and protein synthesis inhibition than is elastase, supporting earlier findings that the production of these two enzymes is regulated independently in the macrophage (11).

Degradation of Extracellular Matrices of Endothelial Cells and Fibroblasts. Cultured endothelial cells produce a basement membrane-like extracellular matrix that contains glycoproteins and collagen but little elastin (12, 13). The digestion of this matrix by macrophages was compared to the digestion of the R22 SMC matrix (Fig. 4). The macrophages degraded the endothelial cell matrix avidly; the rate of degradation was accelerated by Plg, but to a lesser extent than in the SMC matrix. There was significant degradation of the collagen in the endothelial cell matrix.

Degradation of the collagen-rich matrix produced by the fibroblastic line R22 ClF was also studied. The collagen was easily digested by mammalian collagenase (4), and live macrophages degraded the glycoprotein components and small amounts of

collagen (Table V). These observations are consistent with the low collagenase activity secreted by macrophages (3).

Discussion

We have demonstrated that cultured macrophages can degrade the glycoproteins, elastin, and collagens present in insoluble extracellular matrices. Our data obtained with TG macrophages cultured in contact with a vascular connective tissue matrix strongly suggest that removal of matrix glycoproteins is essential for rapid hydrolysis of elastin. Although macrophage elastase was primarily responsible for degradation of elastin (4), macrophage Plg activator generated plasmin which hydrolyzed the glycoprotein components of the matrix, and allowed elastase better access to elastin. Thus, although plasmin has no direct elastinolytic potential, its presence indirectly facilitated the removal of elastin from the extracellular matrix.

These observations may have implications in disease states, such as emphysema, in which breakdown of elastin is thought to be involved. Electron microscopic examination of the elastic fibers present in arteries, lung, and skin (14) and in the SMC matrix (8) shows two distinct components. Elastin, which is the major amorphous component, is surrounded by the distinct (10–12 nm) fibrillar structures of microfibrillar glycoprotein (15–16), which is thought to play a role in the ordered assembly of amorphous elastin during fibrillogenesis (14). Our results suggest that the amorphous elastin in these fibers may be protected from hydrolysis by the presence of the glycoprotein coat. The rate-limiting factor in elastin fiber breakdown in the presence of elastinolytic enzymes may be removal of glycoprotein. Such a role has been suggested for protection of glycosaminoglycans by collagen (17), and of collagen by glycoproteins (18).

The secretion of several neutral proteinases, including Plg activator (1), elastase (2), collagenase (3), and less well-defined proteinases is associated with cell stimulation and phagocytic activity. One of the physiological roles of the macrophage Plg activator may be to utilize the proteolytic potential of Plg to remove insoluble connective tissue glycoproteins. The Plg activator produced by human fibrosarcoma cells may have a similar role (6). Although plasmin has been demonstrated to regulate the collagenolytic potential of rheumatoid synovial cells by activating latent forms of collagenase (19), this mechanism was not evident in matrix degradation by macrophages, even though there are inhibitors and latent forms of the secreted proteinases (4, 19–21).

TABLE V

Degradation of R22 ClF Fibroblastic Matrix by Macrophages

Plg added	Radioactivity solubilized (percent of total)	Matrix component digested	
		Glycoprotein	Collagen
μg/ml		%	
0	6.2	21	7
10	20.0	66	20

TG macrophages (5×10^5/well) were cultured on the R22 ClF matrix labeled with [^{3}H]proline for 48 h. Distribution of the total radioactivity in the matrix was 8% glycoprotein and 92% collagen, as determined by sequential enzyme digestion (4).

These data suggest that, in addition to their structural and organizational roles (18), the connective tissue glycoproteins confer resistance to proteolysis, and that the macrophage Plg activator may play a role in the degradation of the extracellular matrix. Because this degradation is important in many degenerative and invasive diseases, studies on glycoprotein removal from matrix should be of wide significance. In the accompanying report (8) we examine morphologically and biochemically the extracellular, pericellular, and intracellular events involved in the degradation of glycoproteins and elastin by macrophages.

Summary

Thioglycollate-elicited mouse peritoneal macrophages were cultured in contact with the mixture of extracellular matrix proteins produced by rat smooth muscle cells in culture. Both live macrophages and their conditioned media hydrolyzed glycoproteins, elastin, and collagen. Live macrophages also degraded extracellular connective tissue proteins secreted by endothelial cells and fibroblasts.

The glycoproteins in the matrix markedly inhibited the rate of digestion of the other macromolecules, particularly elastin. When plasminogen was added to the matrix, activation of plasminogen to plasmin resulted in the hydrolysis of the glycoprotein components, which then allowed the macrophage elastase easier access to its substrate, elastin. Thus, although plasmin has no direct elastinolytic activity, its presence accelerated the rate of hydrolysis of elastin and therefore the rate of matrix degradation. These findings may be important in an understanding of disease states, such as emphysema and atherosclerosis, that are characterized by the destruction of connective tissue.

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References

1. Unkeless, J. C., S. Gordon, and E. Reich. 1974. Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.* **139**:834.
2. Werb, Z., and S. Gordon. 1975. Elastase secretion by stimulated macrophages. Characterization and regulation. *J. Exp. Med.* **142**:361.
3. Werb, Z., and S. Gordon. 1975. Secretion of a specific collagenase by stimulated macrophages. *J. Exp. Med.* **142**:346.
4. Werb, Z., M. J. Banda, and P. A. Jones. 1980. Degradation of connective tissue matrices by macrophages. I. Proteolysis of elastin, glycoproteins, and collagen by proteinases isolated from macrophages. *J. Exp. Med.* **152**:1340.
5. Banda, M. J., and Z. Werb. Mouse macrophage elastase: purification and characterization as a metalloproteinase. *Biochem. J.* In press.
6. Jones, P. A., and Y. A. DeClerck. 1980. Destruction of extracellular matrices containing glycoproteins, elastin and collagen by metastatic human tumor cells. *Cancer Res.* **40**:3222.
7. Jones, P. A., and T. Scott-Burden. 1979. Activated macrophages digest the extracellular matrix proteins produced by cultured cells. *Biochem. Biophys. Res. Commun.* **86**:71.
8. Werb, Z., D. F. Bainton, and P. A. Jones. 1980. Degradation of connective tissue matrices by macrophages. III. Morphological and biochemical studies on extracellular, pericellular,

and intracellular events in matrix proteolysis by macrophages in culture. *J. Exp. Med.* **152:** 1537.

9. Werb, Z., C. L. Mainardi, C. A. Vater, and E. D. Harris, Jr. 1977. Endogenous activation of latent collagenase by rheumatoid synovial cells. Evidence for a role of plasminogen activator. *N. Engl. J. Med.* **296:**1017.
10. Gordon, S., and Z. Werb. 1976. Secretion of macrophage neutral proteinase is enhanced by colchicine. *Proc. Natl. Acad. Sci. U. S. A.* **73:**872.
11. Werb, Z. 1978. Biochemical actions of glucocorticoids on macrophages in culture. Specific inhibition of elastase, collagenase, and plasminogen activator secretion and effects on other metabolic functions. *J. Exp. Med.* **147:**1695.
12. DeClerck, Y. A., and P. A. Jones. 1980. The effect of ascorbic acid on the nature and production of collagen and elastin by rat smooth muscle cells. *Biochem. J.* **186:**217.
13. Jones, P. A. 1979. Construction of an artificial blood vessel wall from cultured endothelial and smooth muscle cells. *Proc. Natl. Acad. Sci. U. S. A.* **76:**1882.
14. Uitto, J. 1979. Biochemistry of the elastic fibers in normal connective tissues and its alterations in diseases. *J. Invest. Dermatol.* **72:**1.
15. Muir, L. W., P. Bornstein, and R. Ross. 1976. A presumptive subunit of elastic fiber microfibrils secreted by arterial smooth-muscle cells in culture. *Eur. J. Biochem.* **64:**105.
16. Ross, R., and P. Bornstein. 1969. The elastic fiber. I. The separation and partial characterization of its macromolecular components. *J. Cell Biol.* **40:**366.
17. David, G., and M. R. Bernfield. 1979. Collagen reduces glycosaminoglycan degradation by cultured mammary epithelial cells: possible mechanism for basal lamina formation. *Proc. Natl. Acad. Sci U. S. A.* **76:**786.
18. Anderson, J. C. 1976. Glycoproteins of the connective tissue matrix. *Int. Rev. Connect. Tiss. Res.* **7:**251.
19. Werb, Z., and J. Aggeler. 1978. Proteases induce secretion of collagenase and plasminogen activator by fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **75:**1839.
20. Werb, Z. 1978. Pathways for the modulation of macrophage collagenase activity. *In* Mechanisms of Localized Bone Marrow Loss. Special Supplement to Calcified Tissue Abstracts. 213.
21. Nolan, J. C., S. Ridge, A. L. Oronsky, L. L. Slakey, and S. S. Kerwar. 1978. Synthesis of a collagenase inhibitor by smooth muscle cells in culture. *Biochem. Biophys. Res. Commun.* **83:** 1183.