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*

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Macrophages, which secrete proteolytic enzymes capable of hydrolyzing elastin, collagen, and glycoproteins, may play a prominent role in the turnover of connective tissue macromolecules at inflammatory sites. Their degradative capacity has been demonstrated on purified connective tissue substrates (1–6) and on complex extracellular matrices produced by cultured smooth muscle cells (SMC),[1] fibroblastic cells, and endothelial cells (7).

Although macrophages in culture degrade these extracellular matrices to amino acids (8, 9), the precise localization of the matrix solubilization is not known. Endocytosis of insoluble matrix components would be virtually impossible without limited extracellular proteolysis. Understanding of the regulation of this degradation is complicated by the observation that neutral proteinases are secreted by cells along with proteolytic inhibitors (7, 10–12). In addition to extracellular digestion by neutral proteinases and intracellular digestion by lysosomal hydrolases, there is a third location for matrix solubilization at the cell surface and in the pericellular space. This zone differs from other extracellular sites because (a) local saturation of proteinase inhibitors may take place there, (b) cellular metabolism, including lactate production, could lower local pH so that lysosomal hydrolases could participate in matrix degradation, and (c) cell surface-bound enzymes could participate directly in degradation. All three of these are possibilities for the macrophage. At least some fibrinolysis is catalyzed by plasminogen activator in the presence of overwhelming plasma protein inhibitors (13), and it is known that osteoclasts create extracellular pockets where lysosomal enzymes are secreted (14). Lysosomal enzymes are also secreted during phagocytosis (15). Cell surface proteinases and serine esterases have been identified on mononuclear phagocytes (16–19) and have been shown to participate in amyloid degradation and cell movement (18, 19).

The aim of the present study was to determine the localization of macrophage-

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

mediated degradation of matrix proteins. We examined the sites of matrix degradation ultrastructurally, and biochemically monitored the effects of modulation of macrophage secretion, endocytosis, and activity of macrophage hydrolases on matrix degradation.

Materials and Methods

Except where indicated below, all materials and methods used are described in the previous report (7) and the accompanying report (9).

Macrophage Culture Experiments. Mouse peritoneal macrophages elicited with thioglycollate broth (TG) or other inflammatory agents (7) were plated at 2.5 × 10^5 macrophages/cm^2 in culture dishes that contained [^{3}H]proline-labeled complete or glycoprotein-depleted (i.e., trypsin-pretreated) R22 SMC insoluble extracellular matrix (7) in Dulbecco's modified Eagle's medium supplemented with 0.2% lactalbumin hydrolysate. After 2 h, the nonadherent cells were removed and adherent cells were incubated with fresh medium with or without bovine plasminogen (Plg). For experiments with fetal bovine serum (FBS), inhibitors, phagocytic or pharmacological agents, these substances were added to cultures after the initial 2-h adherence period. Prostaglandin E$_2$, dexamethasone, and zymosan were purchased from Sigma Chemical Co., St. Louis, Mo., cytochalasin B from Aldrich Chemical Co., Inc., Milwaukee, Wisc., and latex particles from Dow Diagnostics, Dow Chemical Co., Indianapolis, Ind. Cholesterol-albumin coacervates were prepared as described previously (20). Degradation of matrix was investigated morphologically and by solubilization of radioactive peptides from the matrix and sequential enzyme digestion (7).

For experiments in which matrices were cultured at a distance from macrophages, R22 SMC matrix was prepared on 15-mm plastic cover slips (Thermanox; Lux Scientific Corp., Newbury Park, Calif.) (21) as described for culture dishes (7). Macrophages were then plated on plain plastic cover slips or on cover slips coated with complete or glycoprotein-depleted R22 SMC matrix in medium (1 ml for 24-well plates). Plain or coated cover slips were floated above the cell layers with the matrix side facing the medium.

Transmission Electron Microscopy. Macrophages were cultured on complete or glycoprotein-depleted [^{3}H]proline-labeled R22 SMC matrices prepared in 35-mm dishes. The progress of digestion was monitored at various intervals by the solubilization of radioactivity. Cells and SMC matrix adhering to tissue culture dishes were fixed for 60 min at 22°C in 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, that contained 1% sucrose. They were then washed briefly in the same buffer, post-fixed in buffered 1% OsO$_4$, and stained en bloc with aqueous uranyl acetate. All processing continued until dehydration in absolute alcohol was complete. Propylene oxide (4 ml) was then added to each dish and the surfaces were rinsed vigorously with a Pasteur pipette. As the adherent layer detached, it was transferred to polypropylene tubes, then concentrated into small pellets, and embedded in Epon. Thin sections were stained for 30 min with 1% phosphotungstic acid in deionized water, for 30 min with 5% aqueous uranyl acetate, and for 15 min with alkaline lead citrate.

Scanning Electron Microscopy. Macrophages cultured on SMC matrix prepared on 15-mm plastic cover slips were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 60 min at 22°C, then dehydrated through graded alcohols, critical-point-dried with the use of liquid CO$_2$ in a Bowmar apparatus (Bowmar Instrument Corp., Fort Wayne, Ind.), coated with ~20 nm of gold in a Hummer sputter coater (Technics, Inc., Alexandria, Va.), and examined by means of a Cambridge scanning electron microscope (Cambridge Instrument Co., Inc., Ossining, N. Y.) at 20 kV.

Results

Ultrastructural Analysis of Matrix Degradation. We examined the interaction of TG macrophages with R22 SMC matrix by transmission electron microscopy (Fig. 1 a). The matrix consisted of easily identifiable fibers of collagen, microfibrillar and amorphous components of elastin, and granular material that probably corresponds

FIG. 1. Transmission electron micrograph of a TG macrophage (MAC) that has been allowed to spread on complete R22 SMC matrix for 2 h. (1 a) The lower surface of the macrophages is adherent to the matrix (double-headed arrow), which rests on the surface (S) of the plastic culture dish. The nonadherent plasma membrane has characteristic microvilli (mv), and the cell contains numerous endocytic vacuoles (ev), which are frequently seen in cells elicited with thioglycollate, and mitochondria (m). × 13,500. (1 b) The matrix can be seen at a higher magnification and is oriented from top (upper left corner) to bottom, where a portion of the surface of the culture dish is apparent. Typically, the uppermost layer is granular (arrow) and represents glycoprotein. Typical banded collagen fibers (c) are abundant. There are also mature elastin fibers consisting of microfibrillar glycoprotein (mf) and the amorphous elastin component (e), which are electron dense after staining with phosphotungstic acid. × 42,500.

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to glycoproteins (Fig. 1 b). There were also areas of electron lucency that may represent other matrix material that was not stained with the procedure used here. TG macrophages adhered to the extracellular matrix but did not become as completely spread as they did on plastic.

After 72 h incubation in serum-free medium, much of the granular glycoprotein had disappeared from the matrix (Fig. 2 a); this was reflected in the solubilization of 31% of the [^{3}H]proline radioactivity from the matrix. However, most of the elastin particles and collagen fibers appeared intact. In addition, there was no evidence of intracellular matrix components. The granular glycoprotein material nearest to the macrophages seemed to disappear first.

Matrix degradation proceeded more rapidly in the presence of Plg. At 72 h, 68% of the radioactivity was solubilized from the matrix in this experiment, all of the identifiable glycoproteins had disappeared, and the number, size, and integrity of the elastin particles were reduced considerably (Fig. 2 b). The moth-eaten appearance of the remaining amorphous elastin clearly suggested that these insoluble particles were being digested at an extracellular site. In addition, the macrophages had migrated under the remaining matrix and were adherent to the plastic surface in many places. Much of the collagen was still intact in banded fibers and very small fibrils. Coated vesicles were prominent on the basal surface of macrophages, although these were somewhat obscured by the phosphotungstic acid used to visualize the connective tissue proteins.

In the accompanying report (9) we demonstrate that the rate of degradation of the elastin components of extracellular matrix was accelerated by removal of the matrix glycoproteins by pretreatment with trypsin. The trypsin-treated matrix in Fig. 3 a resembles the matrices digested by macrophages for 72 h (Fig. 2 a) in which glycoproteins had been removed by macrophage-mediated processes. The trypsin-treated matrix contained morphologically intact elastin and collagen but little microfibrillar glycoprotein. The macrophages solubilized 62% of the radioactivity of the trypsin-treated matrix after 72 h incubation in serum-free medium. Much of the morphologically identifiable elastin was removed from the matrix and the size of the remaining particles was reduced (Fig. 3 b). The cells now rested on the surface of the plastic in many places and the majority of the collagen was still intact.

Because of the sampling problems of transmission electron microscopy, it was not feasible to compare matrix that was adjacent to and under the macrophages with matrix that was in the culture but not in contact with cells. Also, cinematography analysis showed that the macrophages migrated over the surface of the matrix: ~50 μm during the 72-h period in serum-free medium and <30 μm in the presence of Plg (R. Glass and Z. Werb. Unpublished observations.). We therefore examined macrophages by scanning electron microscopy to determine whether disappearance of the extracellular matrix was localized near cells. Macrophages were plated on the matrix at 5 × 10^4 cells/cm^2, one-fifth the usual density, so that there would be sufficient cell-free matrix between cells. Under conditions of rapid digestion of the complete matrix in the presence of Plg, two distinct morphological changes in the extracellular matrix were observed at 72 h. At a distance from the macrophages, the matrix appeared more friable than matrix incubated without cells (Fig. 4). A more localized area of matrix digestion in which only amorphous particles of elastin remained was visible near many but not all cells. Residual collagen fibrils were also seen on the surface of
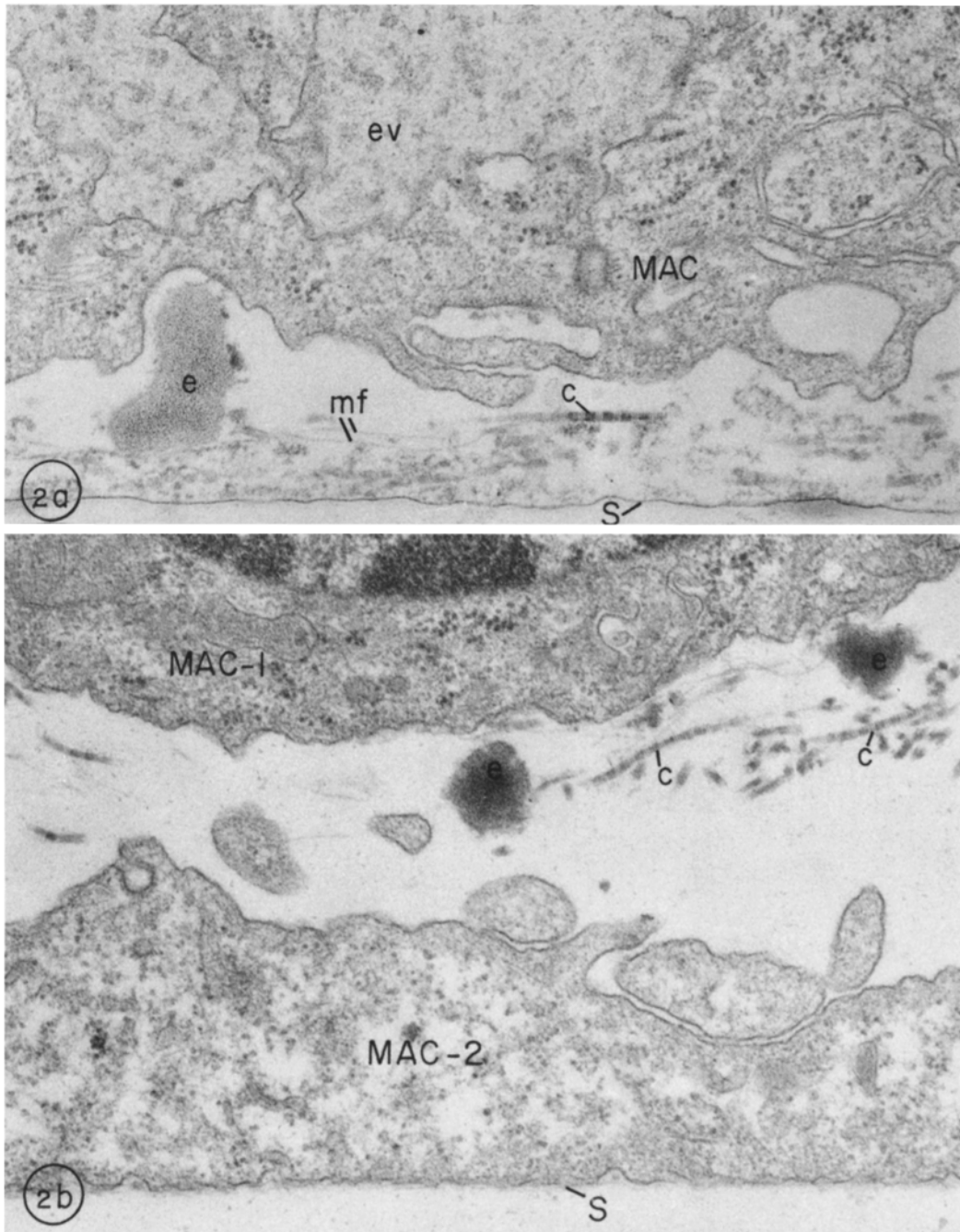
Fig. 2. Transmission electron micrograph of TG macrophages cultured on complete matrix for 72 h. (2a) Macrophages were cultured in serum-free medium. Note that most of the granular and fibrillar glycoprotein material stained with phosphotungstic acid has been removed, but collagen (c), elastin particles (e), and some microfilaments (mf) are still present ($\times$ 41,250). (2b) Macrophages were cultured in medium containing 10 μg Plg/ml. Note that all of the densely staining glycoprotein material has been digested, although collagen fibers (c) still remain. Amorphous elastin (e) appears moth-eaten and particles are reduced in size. Although some macrophages are adherent to matrix (MAC-1), in many places, the macrophages (MAC-2) have pushed their way under the matrix and are now directly adherent to the plastic surface (S). Note the large areas of electron lucency which could denote the presence of other unstained matrix components. $\times$ 40,000.
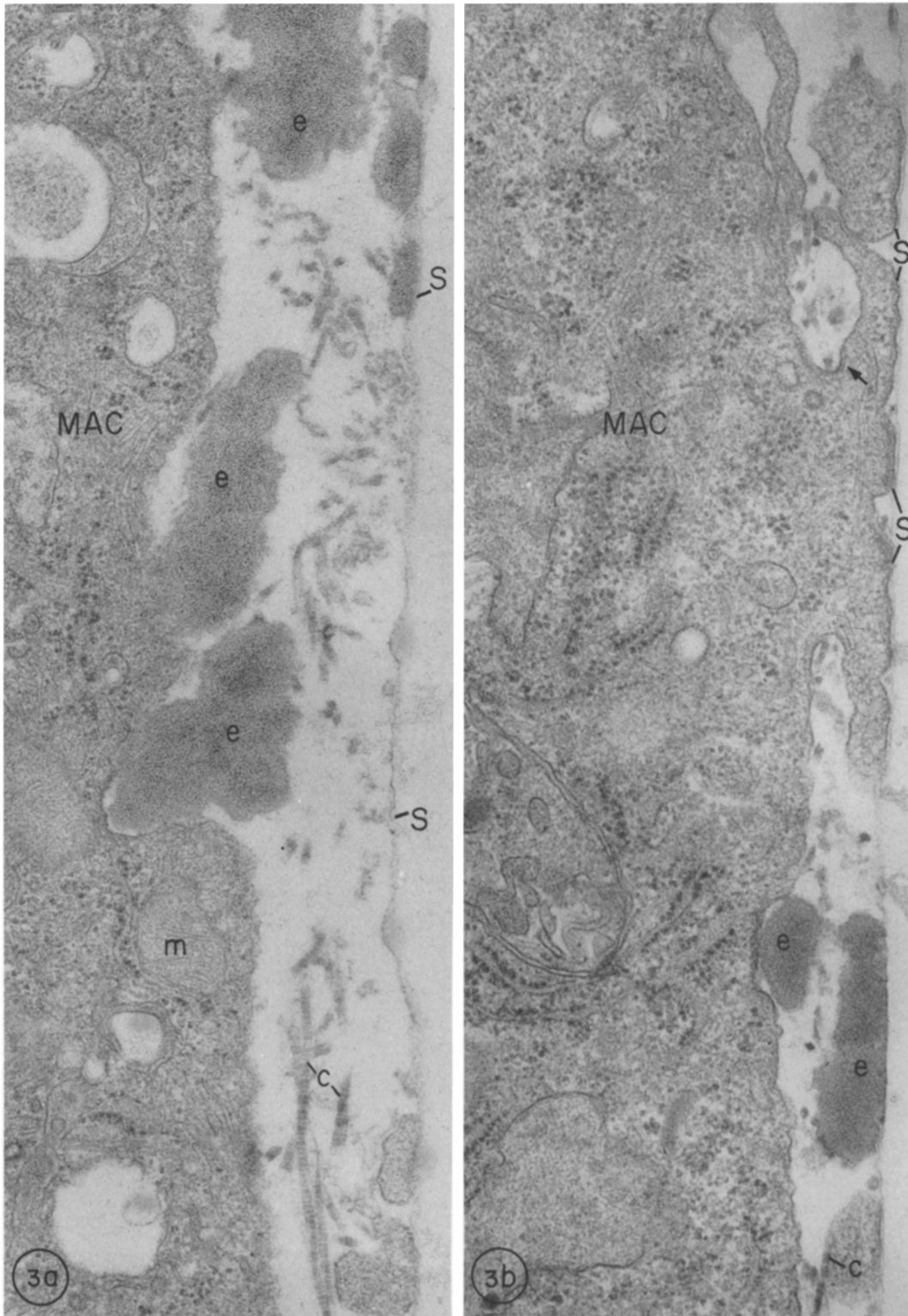
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Fig. 3. Transmission electron micrograph of TG macrophages growing on R22 SMC matrix that has been depleted of glycoproteins by pretreatment with trypsin. (3 a) At 2 h of incubation the macrophage (MAC) is adherent to the matrix, which lacks the densely stained granular and microfibrillar glycoproteins (compare with Fig. 1), whereas the amorphous elastin (e) and collagen components (c) appear unchanged. × 40,000. (3 b) At 72 h of incubation in serum-free medium, the macrophages (MAC) are directly adherent to the surface (S) of the culture dish in many places. The amorphous elastin particles (e) are decreased in number and size, but many banded collagen fibers (c) are still visible. The arrow points to a coated pit. × 40,000.
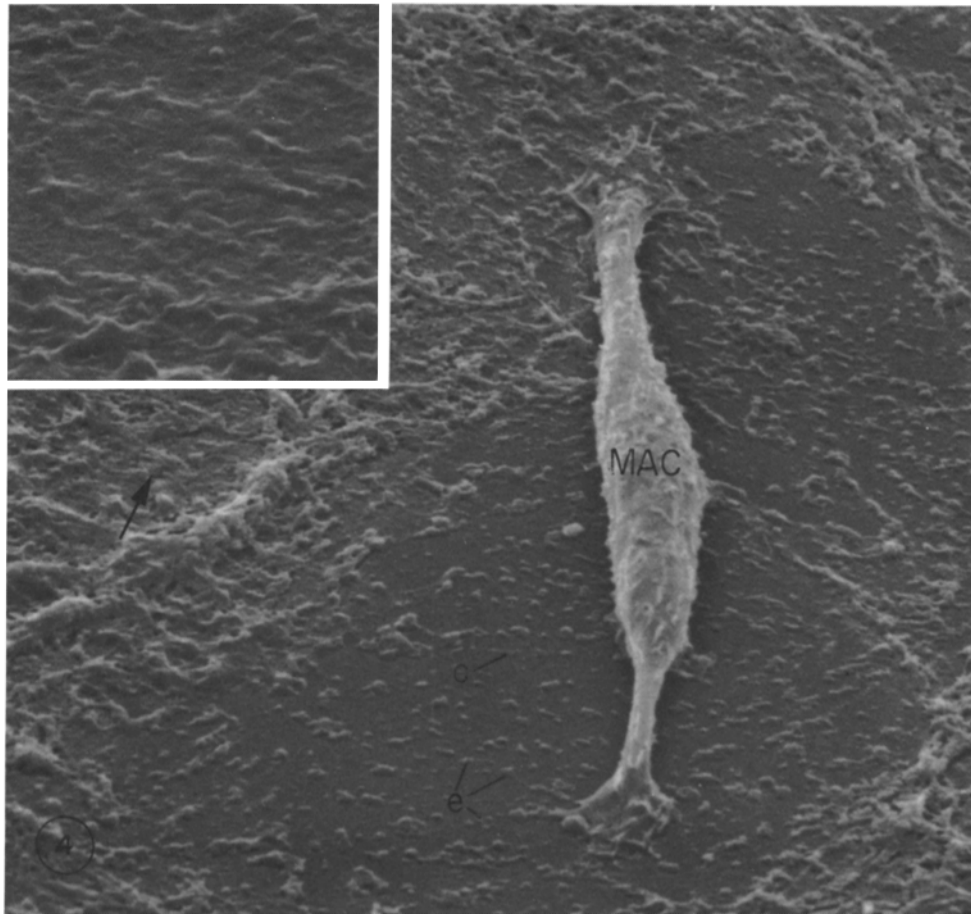
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Fig. 4. Scanning electron micrograph of TG macrophages cultured on complete R22 SMC matrix in serum-free medium in the presence of 10 μg Plg/ml for 72 h. Note the zone of clearing around the macrophage (MAC) that contains only a few amorphous elastin particles (e). Collagen fibrils (c) on the surface of the dish are barely visible at this magnification. At a distance from the cell the matrix has a friable appearance (arrow), as compared with a control matrix (inset at upper left), which was incubated without cells. × 2,850.

the cover slip at higher magnifications. This suggests that degradation may be localized near cells. In the absence of Plg, degradation of matrix by macrophages was less marked and zones of concentrated matrix breakdown were fewer and more limited in size (not shown).

Comparison of Degradation by Macrophages in Contact with and Separated from Matrix. Biochemical data have indicated that the degradation of connective tissue matrices is initially extracellular and is completed within lysosomes (7-9). Even though enzymes produced by macrophages degraded the matrix components as avidly as cultured macrophages (7, 9), our studies with transmission electron microscopy and scanning electron microscopy suggested that some matrix breakdown was limited to the area underneath and in the immediate vicinity of the macrophages. To reconcile these two observations we performed some experiments with macrophages cultured in contact

TABLE I

Comparison of the Rate of Degradation of Extracellular Matrix in Contact with Macrophages or at a Distance from Them

Matrix preparation	Plg added	Ratio of radioactivity solubilized (matrix in contact:matrix at a distance)*
	$\mu g/ml$	
Complete	0	1.06 ± 0.05
	10	1.42 ± 0.50
Glycoprotein-depleted	0	1.24 ± 0.20
	10	4.29 ± 2.32

TG macrophages (5 × 10⁵) were plated on plain 15-mm-diameter plastic cover slips or cover slips that contained [³H]proline-labeled R22 SMC matrix, some of which had been glycoprotein-depleted by pretreatment with trypsin. After 2 h, the cell layers were washed, 1 ml serum-free Dulbecco's modified Eagle's medium with lactalbumin hydrolysate was added with or without Plg, and a second cover slip (plain or with matrix) was floated on the medium. Solubilization of radioactivity was monitored at 24 h and 48 h.
* Results are expressed as mean ± SEM of four separate experiments with different matrix preparations.

with R22 SMC matrix, and others with the matrix floating above the macrophages at a distance of ~2 mm. There was little difference in the rate of degradation of complete matrix under these conditions (Table I). The degradation of the floating matrix was reduced, although somewhat variably, in the presence of Plg. The degradation of the floating matrices depleted of glycoproteins was reduced even more dramatically in the presence of Plg.

Modulation of Endocytosis and Intracellular Digestion. Endocytosis and lysosomal proteolysis are involved in completion of digestion of matrix proteins to amino acids and oligopeptides (7–9). To test whether these two mechanisms are directly involved in degradation of matrix proteins, we selectively inhibited endocytosis and lysosomal proteolysis with cytochalasin B and colchicine. These alkaloids, which decrease phagocytosis (22–24) and enhance the secretion of neutral proteinases by macrophages (9, 25), enhanced overall matrix degradation by macrophages and specifically increased degradation of both glycoproteins and elastin (Fig. 5) in the absence or presence of Plg. Colchicine also increased the rate of degradation of floating matrices by live macrophages (not shown) and degradation of matrix by medium conditioned by macrophages (9).

Primary amines enter lysosomes rapidly, increase lysosomal pH, and inhibit protein turnover mediated by lysosomal proteinases (26). Amines also inhibit uptake of lysosomal hydrolases (27). At concentrations affecting lysosomal functions, NH_4Cl did not reduce the overall rate of matrix degradation by macrophages (Fig. 5). However, the size of peptides present in the medium at 48 h, as determined by solubility in 5% (wt:vol) trichloroacetic acid, was larger in NH_4Cl-treated cultures (11% soluble) than in control (23% soluble). Thus, endocytosis and intracellular digestion did not affect the overall rate of degradation, but had a role in the ultimate fate of the solubilized peptides.

Effects of Proteinase Inhibitors on Matrix Degradation by Macrophages. Specific inhibitors of serine, metallo-, thiol, and carboxyl proteinases (2) were used to determine the role
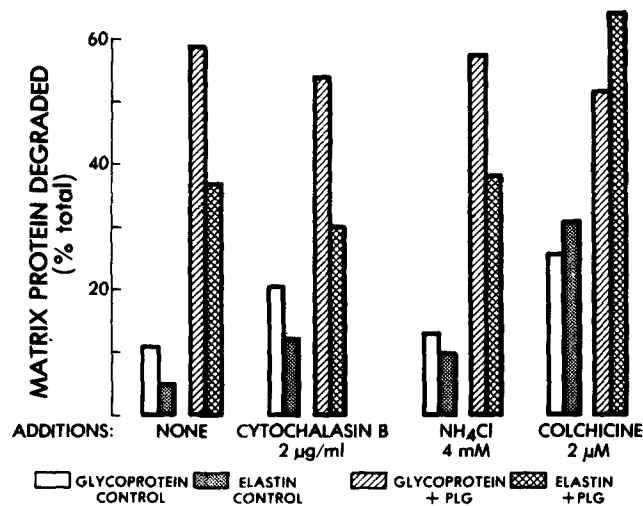
Fig. 5. Effect of modulation of endocytosis and lysosomal digestion on degradation of R22 SMC matrix by TG macrophages. Glycoprotein and elastin components solubilized from the matrix by 5×10^5 TG macrophages at 48 h in the absence or presence of 10 μg Plg/ml and with agents added as indicated at 2 h were determined by sequential enzyme digestion (7). The degradation of collagen was insignificant and is not shown.

TABLE II

Effect of Proteinase Inhibitors on Matrix Digestion by TG Macrophages

Inhibitor added	Amount	Radioactivity solubilized (percent of total)	
		Without Plg	With Plg
None	—	4.5	32.0
FBS	10%	2.5	12.1
α_2-Macroglobulin	25 μg/ml	1.8	10.3
α_1-Proteinase inhibitor	100 μg/ml	5.8	8.3
EDTA	0.5 mM	1.5	17.3
1,10-phenanthroline	0.1 mM	0.9	12.4
Leupeptin	10 μg/ml	4.8	10.9
Pepstatin	1 μg/ml	5.1	30.8

TG macrophages (5×10^6/well) were plated on complete [^{3}H]proline-labeled R22 SMC matrix with added inhibitors in the absence or presence of 10 μg bovine Plg/ml. Radioactivity solubilized was measured at 48 h.

of various proteinases in matrix degradation by live macrophages. Although serum is rich in proteinase inhibitors, matrix degradation was only partially inhibited by FBS in the absence or presence of added Plg (Table II). Less degradation was seen in the absence of Plg. One striking difference in matrix degradation by macrophages in the presence of FBS was the nature of the matrix components degraded. Cells in serum-free medium degraded 11% of the glycoproteins and 8% of the elastin without Plg, and 59% and 37% with Plg, respectively. In contrast, cells in FBS degraded 12% of the glycoproteins and none of the elastin without Plg, and 50% of the glycoproteins and none of the elastin with Plg. These data suggest that the macrophage elastase was

effectively inhibited by inhibitors in FBS, but that some Plg activator remained active, possibly in pericellular locations. When colchicine was added to macrophage cultures to accelerate the secretion of elastase and the degradation of elastin, 10% FBS reduced the degradation of elastin from 55% to 6% of the total and glycoprotein from 74% to 31%.

α_2-Macroglobulin, an inhibitor of all endopeptidases, and α_1-proteinase inhibitor, an inhibitor of serine proteinases including plasmin (28), also decreased matrix degradation (Table II). EDTA and 1,10-phenanthroline, inhibitors of metalloproteinases such as elastase, decreased matrix degradation in the absence of Plg. Overall matrix degradation was decreased somewhat in the presence of Plg, although this decrease corresponded to decreased degradation of elastin, but not of glycoprotein. Pepstatin, an inhibitor of lysosomal cathepsin D, did not affect matrix degradation. Leupeptin, an inhibitor of thiol proteinases such as cathepsin B and of some serine proteinases including plasmin, decreased degradation of complete matrix in the presence of Plg, presumably because it inhibited extracellular plasmin (Table II).

Degradation of Matrix by Macrophages Elicited by Various Stimuli. Proteinases are secreted at different rates, and in varying ratios, by macrophages elicited by different types of inflammatory stimuli administered in vivo (7, 29). These macrophages also differ in endocytic activity, although their content of lysosomal hydrolases is similar (7). We therefore compared these inflammatory macrophages in their capacity to degrade extracellular matrices. Resident and endotoxin-elicited macrophages degraded the matrices most slowly (Table III). Pyran copolymer-elicited macrophages, which secrete abundant Plg activator but little elastase, degraded complete matrix poorly without added Plg and was unable to degrade matrix depleted of glycoproteins. Periodate-elicited and TG macrophages and the P388D1 macrophage line degraded glycoprotein and elastin components of the matrix, reflecting their high rates of secretion of elastase and Plg activator.

Effect of Pharmacological Agents on Enzyme Secretion and Matrix Degradation. To further localize the sites of matrix degradation, we tested pharmacological agents that affect secretion of neutral proteinases during degradation of connective tissue proteins by live TG macrophages. The extent of matrix degradation and the amount of secreted macrophage enzymes remaining in the supernate at the end of the experiment were measured (Table IV). One new finding emerging from this protocol was that the amount of elastase present in the conditioned medium that contained Plg was reduced, presumably because of degradation of elastase by plasmin. Colchicine, which increased secretion of elastase but decreased secretion of Plg activator in this experiment, stimulated the rate of matrix degradation. Dexamethasone, a glucocorticoid that reduces proteinase secretion by macrophages (30), decreased matrix degradation in the absence and presence of Plg. Prostaglandin E_2 and endotoxin, which decreased secretion of Plg activator while slightly increasing secretion of elastase, did not alter matrix degradation appreciably, suggesting that the amount of Plg activator was not limiting in these reactions.

Effects of Phagocytic Stimuli on Matrix Degradation. Endocytosis is an important function of inflammatory macrophages that triggers secretion of Plg activator, elastase, lysosomal enzymes, and prostaglandins, in addition to conveying substances to macrophage lysosomes (3, 13, 15, 31). We examined the effects of endocytosis and related processes on matrix degradation mediated by endotoxin-elicited macrophages

TABLE III

Degradation of Extracellular Matrix Proteins by Macrophages in Various States of Stimulation

Eliciting agent	Plg added	Complete matrix degraded (percent of total)			Glycoprotein-depleted matrix degraded (percent of total)	
		Glycoprotein	Elastin	Collagen	Elastin	Collagen
	$\mu g/ml$					
Resident	0	8	1	0	7	0
	10	12	13	1	12	0
Endotoxin	0	7	2	0	9	4
	10	26	11	1	8	6
Periodate	0	34	9	0	ND*	ND
	10	67	53	0	ND	ND
Thioglycollate	0	38	17	0	31	2
	10	67	46	0	49	10
Pyran copolymer	0	6	4	0	0	0
	10	66	4	0	0	0
P388D1	0	54	28	2	64	17
	10	92	81	12	72	14

Macrophages (5×10^5/well) were plated on [^{3}H]proline-labeled R22 SMC matrix that was complete or depleted of glycoproteins by pretreatment with trypsin (7), in the absence or presence of bovine Plg. After 48 h, the macrophages were lysed with NH$_4$OH and the matrix components that had been degraded were determined by sequential enzyme digestion (7).

* ND, not determined.

(Table V). These cells degraded matrix poorly by themselves, but their rate of matrix degradation was accelerated by phagocytosis of latex particles, zymosan, and cholesterol-albumin complexes. Endotoxin, which is also particulate, did not accelerate the rate of degradation, and none of the phagocytic stimuli were as potent as colchicine in stimulating degradation of matrix. These agents induced degradation of both glycoproteins and elastin, as shown by comparing degradation of complete and glycoprotein-depleted matrices. Addition of phorbol diesters, which stimulate Plg activator secretion (32), also enhanced matrix degradation.

Discussion

The data presented in this paper demonstrate that live macrophages degrade connective tissue macromolecules by circumscribed extracellular proteolysis followed by degradation of fragments to amino acids and oligopeptides within lysosomes. This conclusion is supported by morphological observation of the dissolution of the insoluble matrix components, and biochemical demonstration of significant matrix degradation with millimeter distances separating cells from the connective tissue proteins. In addition, macrophages that secreted neutral proteinases at different rates,

TABLE IV

Effect of Pharmacological Agents Influencing Secretion of Proteinases by TG Macrophages During Matrix Degradation

Agent	Amount	Plg added	Secreted enzymes		Radioactivity solubilized (per-cent of total)
			Elastase	Plg acti-vator*	
		μg/ml	*U/well*		
None	—	0	6.5	15.5	3.6
		10	2.0	23.4	33.9
Colchicine	1 μM	0	36.7	5.9	7.4
		10	10.2	16.2	58.2
Dexamethasone	1 μM	0	4.2	0.7	2.6
		10	2.5	0.2	9.4
Prostaglandin E2	1 μM	0	8.3	10.3	3.7
		10	4.5	6.2	24.6
Endotoxin	10 μg/ml	0	11.9	4.7	6.4
		10	2.9	6.7	43.5

TG macrophages (5 × 10^5/well) were cultured on complete [^{3}H]proline-labeled R22 SMC matrix with added agents as indicated, in the absence or presence of bovine Plg. Radioactivity solubilized was monitored at 46 h, and aliquots of the supernatant medium were then assayed for elastase and Plg activator (7). Elastase assays contained sodium dodecyl sulfate (7).

* Samples from wells that contained Plg were assayed for direct fibrinolytic activity without additional Plg.

TABLE V

Effect of Phagocytic Stimuli and Other Agents on Matrix Degradation by Endotoxin-Elicited Macrophages

Agent	Amount	Radioactivity solubilized (percent of total)			
		Complete matrix		Glycoprotein-depleted matrix	
		Without Plg	With Plg	Without Plg	With Plg
None	—	0.5	4.1	1.2	1.0
Latex particles	50 μg/ml	4.0	14.6	3.6	3.1
Zymosan	25 μg/ml	6.3	16.1	5.7	4.8
Cholesterol-albumin coacervates	100 μg/ml	4.4	8.1	ND*	ND
Colchicine	1 μM	3.0	39.1	25.8	29.0
Endotoxin	10 μg/ml	1.0	2.0	0.6	1.6
12-O-Tetradecanoyl phorbol-13-acetate	20 ng/ml	1.1	16.7	ND	ND

Endotoxin-elicited macrophages (5 × 10^5/well) were grown on [^{3}H]proline-labeled R22 SMC matrices that were complete or glycoprotein-depleted by pretreatment with trypsin (7) in the absence or presence of 10 μg bovine Plg/ml and various agents as indicated. Radioactivity solubilized was measured at 48 h.
* ND, not determined.

as a result of differentiation along different pathways, degraded the matrix proteins in corresponding proportions.

We have also presented several lines of evidence that a portion of the macrophage-mediated degradation of the extracellular matrix is restricted to the pericellular zone of the macrophage. Although the degradative potential of medium conditioned by macrophages was similar to that of cultured cells, scanning electron microscopy revealed some localized degradation near macrophages. These conclusions are supported by experiments indicating that matrix degradation took place slightly more rapidly when cells were in contact with the matrix than when the matrix was separated from the macrophages. Because local control is an important factor in matrix degradation by macrophages in culture, where the extracellular fluid is in excess, it is likely that it plays an even more significant role in vivo.

A significant amount of proteinase secretion by macrophages probably takes place at the basal surface directly onto the matrix. Certainly, the surface of the macrophage in contact with the substrate is active in endocytosis, and possibly in membrane recycling, as judged by the prevalence of clatharin-coated vesicles and coated strips of membrane (Fig. 3; and J. Aggeler, Z. Werb, and J. Heuser. Unpublished observations.), which are thought to mediate some of these processes (33). Some of these sites of secretion may be relatively inaccessible to endogenous and exogenous macromolecular inhibitors of proteinases. Thus, α_2-macroglobulin completely inhibited matrix degradation by macrophage elastase (7) but only partially inhibited degradation by live cells. The macrophages were also less mobile and more closely adherent to matrix in the presence of Plg (9) because plasmin, like trypsin (34) and activated factor B (Bb) of the properidin pathway (35), induces macrophage spreading. It is possible that this close proximity of the basal surface of the macrophages to the matrix contributed to the increased rate of degradation with added Plg. We predict that the immobilizing of macrophages by adherence to insoluble immune complexes, such as those on connective tissue in vasculitis and glomerulonephritis, might accelerate the rate of degradation of extracellular matrix even more. Indeed, neutral proteinases such as elastase, which degrade immunoglobulins (1), may be secreted onto immune complexes and, in addition to degrading the native matrix proteins, may disengage the macrophages bound via their Fc receptors.

Membrane-bound proteinases may also have played a prominent role in producing the circumscribed area of degradation seen with live macrophages. Human, guinea pig, and mouse mononuclear phagocytes have tightly bound neutral proteinases (16–19) and peptidases (36) on their plasma membranes. At least one of these enzymes, factor B (16), is also secreted by macrophages (37, 38), and the cationic properties of macrophage Plg activator and elastase make it probable that they bind to the cell surface to some extent. The relative ineffectiveness of inhibitors of protein and RNA synthesis in abolishing Plg-dependent matrix degradation (9) suggests that membrane-bound Plg activator may be present.

Macrophage elastase also binds tightly to elastin (3). Pericellular degradation may take place because elastase binds to matrix and is trapped there before diffusing into the large excess of culture medium. Localized extracellular degradation of insoluble elastin particles in vivo may be mediated by trapping of degradative enzymes (5, 6).

We obtained little evidence for participation of mouse macrophages in degradation of the cross-linked collagens of the SMC matrices. It is possible that the low amounts

of collagenase secreted by stimulated macrophages (4), coupled with the inaccessibility of the collagen fibers in the matrix (9), minimized the collagenolytic function of these cells. In addition, the collagen degradation measured was sporadic and varied from experiment to experiment, suggesting that most of the collagen degradation could be the product of collagenases secreted by fibroblastic cells that contaminate inflammatory exudates (39). It should be emphasized, however, that on the larger time scales seen in vivo, macrophage participation in collagenolysis could be significant.

Our data identified roles for macrophage elastase and Plg activator in matrix degradation. However, macrophages secrete other proteinases, including complement and lysosomal enzymes (31, 37–39). The present experiments were not sufficiently specific or sensitive to determine whether the proteinases other than elastase, collagenase, and Plg activator had minor contributions to matrix proteolysis. It is unlikely that secreted lysosomal enzymes participated because of their low pH optimum (7) and the lack of effect of specific inhibitors of cathepsins B and D.

We have implicated lysosomal hydrolases in completion of proteolysis of connective tissue proteins to amino acids and oligopeptides (7–9). In the present report, we demonstrate that lysosomal processing was not rate-limiting in solubilization of the cross-linked SMC matrix. Inhibition of endocytosis and lysosomal digestion did not retard the solubilization of the matrix, and the rate of solubilization of matrix proteins by inflammatory macrophages having similar rates of endocytosis differed considerably, correlating instead with their rates of secretion of neutral proteinases. The degradation rates in culture have a bias toward the secreted proteinases because of the excess volume of culture medium. In vivo, the lysosomal functions may be more important because fragments of connective tissue proteins, once dislodged, may be ingested more rapidly.

Chronic inflammatory lesions contain widely varying proportions of mononuclear phagocytes and lymphocytes. The macrophages from these inflammatory sites have specific characteristics depending on the eliciting stimulus (29) and the length of time since their mobilization from the blood (40). Macrophages from lymphocyte-rich lesions induced by microorganisms are characterized by their microbicidal and cytolytic properties (29, 41, 42), and cells from lesions induced by indigestible materials such as carrageenan and thioglycollate, are characterized by their secretion of enzymes that degrade connective tissue proteins (3, 4, 7, 15, 36). In this study, we observed marked differences in the matrix-degrading capacity of mouse macrophages elicited by different stimuli. The cytolytic macrophages elicited with pyran copolymer, a material similar in action to *Corynebacterium parvum* (43), secreted little elastase and degraded matrix poorly. Addition of Plg to these macrophages enhanced their ability to degrade only the glycoproteins of the matrix. These cells did not secrete an enzyme similar to the casein-degrading proteinase reported in some cytotoxic macrophages (41). Macrophages elicited with thioglycollate or periodate were efficient in degradation of elastin and glycoproteins. Endotoxin-elicited macrophages degraded little matrix unless triggered by endocytosis of particles such as latex and cholesterol-albumin coacervates, or by pharmacological agents such as colchicine and phorbol diesters. These data emphasize that inflammatory macrophages are heterogeneous in their ability to mediate connective tissue breakdown. The effectiveness of agents such as endotoxin, glucocorticoids, and prostaglandins in modulating these macrophage properties may also vary with the particular macrophage population.

The mechanisms regulating the macrophage-mediated matrix degradation may be generally applicable to connective tissue degradation by other cells, such as fibroblasts. However, our study implicates both secreted and cell-associated proteinases in complete degradation of matrices at a distance from the cells, at the cell surface, and within the cells, whereas others have observed that human tumor cells invade and digest endothelial and SMC matrices by mechanisms independent of secreted enzymes (21).

Summary

We have shown that macrophages in culture degrade the glycoproteins and amorphous elastin of insoluble extracellular matrices. Ultrastructural observation of the macrophage-matrix interaction revealed that connective tissue macromolecules were solubilized from the matrix extracellularly. At least part of the matrix breakdown was localized to the immediate vicinity of the cells, as shown by morphological and biochemical studies, although the rate of degradation correlated closely with the secretion of elastase and plasminogen activator by the macrophages. Modification of the secretion of proteinases by various inflammatory stimuli in vivo, by glucocorticoids, prostaglandin E_2 or colchicine, or by phagocytosis of latex, zymosan, or cholesterol-albumin complexes in culture was reflected in altered rates of glycoprotein and elastin degradation by the macrophages. Alteration of endocytosis and lysosomal digestion by cytochalasin B, NH_4Cl, and proteinase inhibitors did not decrease the overall rate of matrix solubilization, but reduced the processing of the matrix fragments to peptides.

Therefore, extracellular, pericellular, and lysosomal events each contribute to degradation of extracellular matrix macromolecules by inflammatory macrophages.

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