Clinical Science and Molecular Medicine (1978), **54**, 233–240

The degradation of human glomerular basement membrane with purified lysosomal proteinases: Evidence for the pathogenic role of the polymorphonuclear leucocyte in glomerulonephritis

M. DAVIES, A. J. BARRETT, J. TRAVIS, E. SANDERS AND G. A. COLES

K.R.U.F. Institute of Renal Disease, Welsh National School of Medicine, Royal Infirmary, Cardiff, Wales and Molecular Pathology Department, Strangeways Research Laboratory, Cambridge, U.K.

(*Received* 20 *April* 1977; *accepted* 1 *September* 1977)

Summary

1. Human polymorphonuclear leucocyte elastase and cathepsin G were incubated with preparations of isolated human glomerular basement membrane at neutral pH and 37°C.

2. The ability of these enzymes to degrade glomerular basement membrane was followed by the release of hydroxyproline. Both proteinases released considerable amounts of hydroxyproline.

3. By using Sephadex G-100 it was shown that the solubilized basement membrane fragments appeared as a single peak and had a molecular weight of over 100 000. These proteins after reduction were analysed by sodium dodecyl sulphate-gel electrophoresis to examine their subunit pattern and determine their molecular size.

4. The released basement membrane proteins gave at least four precipitin lines with a rabbit anti-(glomerular basement membrane) antiserum.

5. These results support the concept that polymorphonuclear leucocyte neutral proteinases play an important role in the pathogenesis of glomerulonephritis.

6. At acid pH values cathepsin B also released hydroxyproline from human glomerular basement membrane but the lysosomal carboxyl proteinase, cathepsin D, had no action.

Correspondence: Dr M. Davies, K.R.U.F. Institute of Renal Disease, Royal Infirmary, Newport Road, Cardiff, Wales CF2 1SZ, U.K.

Key words: basement membrane, cathepsins, elastase, glomerulonephritis, leucocytes, proteinases.

Abbreviations: GBM, glomerular basement membrane; SDS, sodium dodecyl sulphate.

Introduction

At least two types of immunologically mediated glomerular disease have been discovered by animal studies (Dixon, 1968). One is induced by deposition within the glomeruli of immune-complexes formed in the circulation, and the other by anti-(glomerular basement membrane) antibodies. Damage to the glomerular basement membrane (GBM) in experimental anti-GBM antibodies disease may occur through fixation of antibody to the basement membrane, which is followed by activation and binding of complement; this causes the chemotactic attraction of polymorphonuclear leucocytes, which in turn selectively release their lysosomal enzymes which degrade the GBM, so that fragments can be detected in the urine together with neutrophil-derived enzymes. It is likely that a similar mechanism is responsible for the renal damage in acute forms of immune-complex disease, although this is not as well documented.

It is now recognized that both types of immunological injury occur in man, with the immune-complex type accounting for most cases of human glomerulonephritis (Dixon, 1968). Some patients with this condition have been shown to have leucocytes in their glomeruli. However, there has been no direct evidence to implicate leucocyte enzymes in the pathogenesis of human renal disease. Sanders, Coles & Davies (1976) have recently demonstrated that patients with rapidly progressive proliferative glomerulonephritis excrete a neutral proteinase together with fragments of GBM in their urine. This enzyme appears to possess many of the properties of polymorphonuclear leucocyte elastase (EC 3.4.2.1). It is therefore important to know whether this elastase can degrade GBM. To answer this question we have studied the effect of incubating highly purified leucocyte elastase with human GBM at neutral pH. We have also investigated another neutral proteinase found in human polymorphonuclear leucocytes, cathepsin G (EC 3.4.21.20) and in addition two lysosomal acid proteinases found in human kidney.

Materials and methods

Human glomerular basement membrane

This was isolated from normal kidneys obtained at autopsy, by the method of Krakhower & Greenspon (1951) as modified by Spiro (1967). In our isolation procedure *p*-mercuribenzoate (28 μmol/l) was included in the sodium phosphate (10 mmol/l) buffer/NaCl (150 mmol/l), pH 7·2 (phosphate-buffered saline), to minimize any degradation of basement membrane by endogenous thiol-dependent proteinases. The isolated glomeruli were suspended in isotonic sodium chloride solution, centrifuged at 600 *g* for 15 min and the supernatant was discarded. This procedure was repeated five times.

Basement membrane was obtained from the glomeruli by ultrasonic disruption. The glomeruli were suspended in 25 ml of NaCl (1 mol/l) in a McCartney bottle immersed in a salt/ice mixture. A MSE Sonicator (model 500) with a stainless-steel probe was tuned to give maximum sonication at setting 10 and used at this setting in 1 min bursts. The suspension was cooled between each burst. The extent of glomerular disruption was followed microscopically. Almost complete disruption was achieved after eight bursts. The sonicated material was washed five times in NaCl (1 mol/l) and finally three times with distilled water. The final preparation was suspended in 20 ml of water and freeze-dried. It was free of DNA and RNA and contained no proteolytic activity at acid or neutral pH.

Antiserum to human GBM was raised in New Zealand White rabbits. Before use the antiserum was absorbed with normal human serum. Double diffusion was performed in 1% agarose (2 mm well size) in sodium phosphate buffer (50 mmol/l), pH 7·0, at room temperature for 24 h.

Human lysosomal elastase and cathepsin G

These were prepared by the method of Baugh & Travis (1976) modified in that a NaCl gradient to 1 mol/l was used to elute cathepsin G from the carboxymethylcellulose column. Cathepsins B and D were prepared as described by Barrett (1973).

The degradation of glomerular basement membrane by lysosomal proteinase was assayed in mixtures (500 μl) containing 2 mg of glomerular basement membrane, Tris/HCl buffer (200 mmol/l), pH 7·4, and various amounts of enzyme (see the Results section). The tubes were rotated in a roller rocker and maintained at 37°C for 18 h. After the incubation period the tubes were centrifuged at room temperature and the supernatant was carefully removed. Samples of this supernatant were hydrolysed in HCl (6 mol/l) for 20 h in sealed glass ampoules before analysis for hydroxyproline. Samples of the supernatant were also examined by sodium dodecyl sulphate (SDS) polyacrylamide-gel electrophoresis. The pellet remaining after the removal of the supernatant was washed once with 500 μl of distilled water and centrifuged. The supernatant was discarded and the pellet resuspended in 500 μl of distilled water. Portions (350 μl) were hydrolysed with HCl (6 mol/l) as above for hydroxyproline determination. The remainder was used for SDS-polyacrylamide-gel electrophoresis. The results were expressed as the amount of hydroxyproline made soluble over the incubation period (i.e. hydroxyproline present in the supernatant divided by hydroxyproline present in the supernatant plus pellet, expressed as a percentage).

Hydroxyproline determinations were carried out as described by Burleigh, Barrett & Lazarus (1974), except that the incubation at 65°C was for 15 min.

SDS-polyacrylamide-gel electrophoresis was carried out in 7·5% gels prepared in Tris (0·1 mol/l)–Bicine buffer, pH 8·1. Samples were incubated at 100°C for 5 min in 1% SDS/Tris (0·1 mol/l)–Bicine buffer (pH 8·1)/2-mercaptoethanol (0·43 mol/l), 20% (2·73 mol/l) glycerol/0·1% Bromophenol Blue; portions (25–50 μg) of the mixture were applied to the gels. Human GBM was solubilized by incubation at 50°C for 60 h. The gels were stained for protein with 0·1% Coomassie Brilliant Blue R250 as described by Starkey & Barrett (1976).

Enzyme assays

Human polymorphonuclear leucocyte neutral proteinase activity. This was measured in leucocytes from the peripheral blood of two healthy donors, obtained by dextran sedimentation and centrifugation on a Hypaque Ficol gradient (Böyum, 1968). The leucocyte preparation was washed with cold 0·2% NaCl to lyse contaminating erythrocytes and 1·6% NaCl was added to restore isotonicity. The cells were centrifuged again and the above procedure was repeated until no erythrocytes remained. Leucocyte granules were prepared and extracted as described by Baugh & Travis (1976).

Total neutral proteolytic activity. This was determined with azocasein as substrate (Starkey & Barrett, 1976), except that a temperature of 45°C was used. Under the conditions of the assay ΔA_{366} was linear with time up to 30 min and linear with enzyme concentration of up to 0·35 unit. One unit of activity is defined as the amount of enzyme which would have given ΔA_{366} of 1·0 in 30 min at 45°C, if the response were linear to this value.

Elastase and cathepsin activity. Elastase activity was measured against *N*-benzyloxycarbonyl-L-alanine 2-naphthyl ester (kindly given by Dr C. G. Knight, Strangeways Research Laboratory, Cambridge, U.K.) and cathepsin G (chymotrypsin-like enzyme) against *N*-benzoyl-DL-phenylalanine 2-naphthyl ester (Starkey & Barrett, 1976). Activities for elastase and cathepsin G were first calculated in nkat (i.e. activity hydrolysing 1 nmol of substrate/s) and then converted into equivalent azocasein units on the basis of the values for the pure enzymes given by Starkey & Barrett (1976). Cathepsin B (EC 3.4.22.1) was assayed with α-*N*-benzoyl-DL-arginine-2-naphthylamide HCl (Barrett, 1976).

Results

Degradation of human glomerular basement membrane by leucocyte neutral proteinases

Since collagen is a major structural component of glomerular basement membrane, the release of hydroxyproline was used as a convenient marker of membrane digestion. The effect of leucocyte elastase on human glomerular basement membrane in suspension was investigated over a range of enzyme concentrations. The release of hydroxyproline increased with amount of enzyme (0·5–2·0 units) up to 70% of the available hydroxyproline (Fig. 1*a*). Cathepsin G also caused the release of hydroxyproline but not to the same extent as that achieved with elastase (Fig. 1*a*). In a separate experiment human basement membrane was incubated with elastase (0·25 unit/mg) and with cathepsin G (0·5 unit/mg of GBM) over a period of 90 h. Under these conditions elastase released 93% of the total hydroxyproline. Efforts to release the remaining hydroxyproline with fresh enzymes were unsuccessful. Under the same conditions cathepsin G released 81% of the available hydroxyproline.

Behaviour of reaction products on gel filtration

An attempt was made to characterize the products made soluble by elastase and cathepsin G by separation on a column of Sephadex G-100. It was found that the elution profiles (Fig. 2), characterized by their absorbance at 280 nm and hydroxyproline content, from both experiments were almost identical (described in Table 1). The high-molecular-weight component (fraction 1) eluted in the void volume and contained hydroxyproline. A second fraction (fraction 2) containing hydroxyproline eluted before some low-molecular-weight proteins. Fraction 1 from both experiments was analysed by SDS-gel electrophoresis. Tubes which contained hydroxyproline were pooled, dialysed against distilled water and freeze-dried. The material was redissolved in distilled water, reduced with 2-mercaptoethanol and then examined by SDS-gel electrophoresis. The electrophoresis patterns of fraction 1 were compared with that of whole glomerular basement membrane (Fig. 3). Comparisons of the gels showed a heterogeneous distribution of proteins with respect to molecular weight. Similar results were obtained with the elastase and cathepsin G digestion products. Proteins with molecular weights of less than 100 000 were noted.

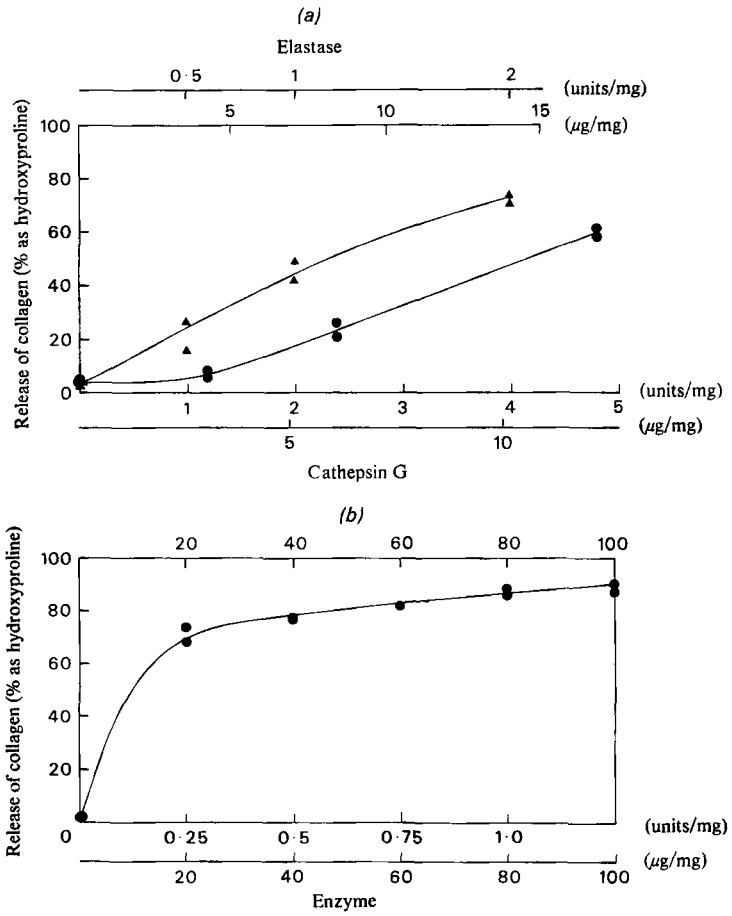

FIG. 1. (*a*) Degradation of human glomerular basement membrane by lysosomal elastase (▲) and cathepsin G (●) at pH 7·5, 37°C for 18 h; results of individual experiments are shown. (*b*) Degradation of human GBM by human cathepsin B at pH 4·0, 37°C for 18 h. Symbols represent results from individual experiments.

Immunodiffusion studies

For the immunochemical characterization of reaction products from GBM digestion a rabbit anti-GBM serum was used. Gel diffusion analysis showed at least four precipitation lines in the final supernatant (Fig. 4). Fraction 1 also yielded several lines but fraction 2 did not appear to possess antigenic properties.

Human leucocyte proteolytic activity (*Table* 2)

Assays with azocasein indicated considerable neutral protease activity in human polymorphonuclear leucocyte azurophil granules (21·4 units/10^9 leucocytes). This proteolytic activity comprised at least two distinct neutral proteases. The activity against a low-molecular-weight substrate for elastase was 3·5 units/10^9 leucocytes, and for cathepsin G was 8·1 units/10^9 leucocytes. In our experiments we failed to detect cathepsin B activity in polymorphonuclear leucocytes.

Effects of incubating purified cathepsin B with human GBM

Purified human cathepsin B released hydroxyproline into the medium upon incubation at 37°C and pH 4·0, as shown in Fig. 1(*b*). Up to 90% of the total hydroxyproline was released over the 18 h incubation period, when the reaction mixture contained 1·25 units of cathepsin B. When the same amount of elastase was used in the incubation mixture about 45% of hydroxyproline was released, and with cathepsin G about 5% (Fig 1*a*).

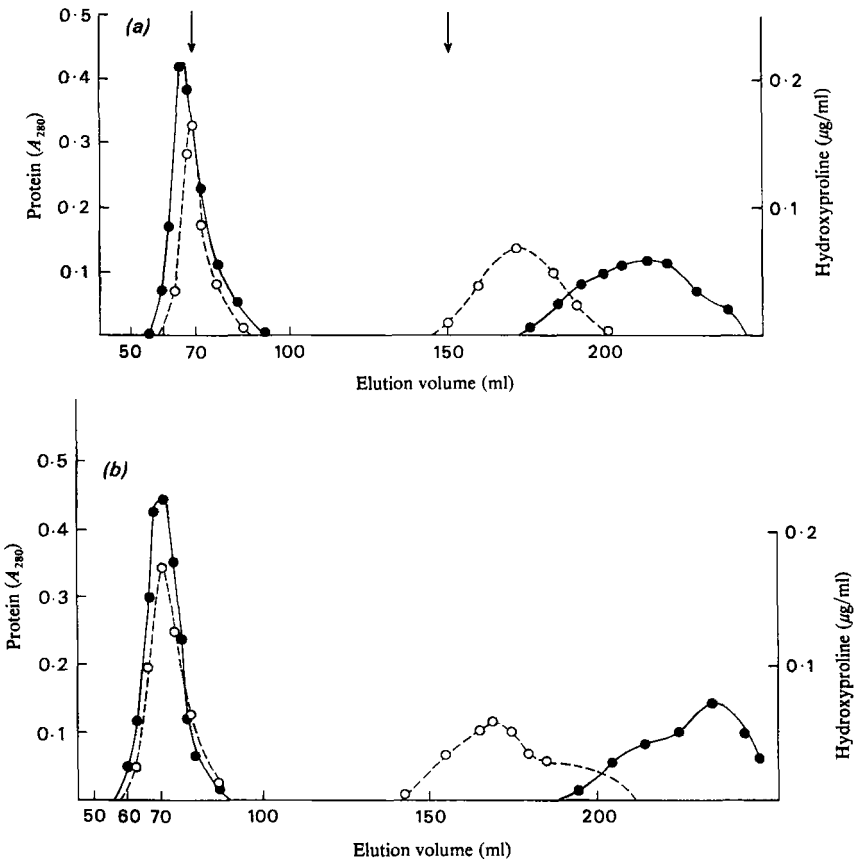

FIG. 2. Gel filtration on a Sephadex G-100 column (2·5 cm × 40 cm) of undialysed material of human GBM obtained by digestion with (*a*) elastase and (*b*) cathepsin G. The column was eluted with Tris–HCl buffer, pH 7·4 (0·2 mol/l), and the fractions were monitored by measuring A_{280} (●, protein) and the hydroxyproline content (○, collagen). In (*a*) the left-hand arrow represents the void volume and the right-hand arrow represents the elution volume for trypsin (mol. wt. 24 000).

TABLE 1. *Degradation of human glomerular basement membrane by neutral proteases*

Enzyme	Time (h)	Activity (unit/mg of GBM)	Hydroxyproline released (μg; % of total)
Elastase	90	0·25	37·02 (93%)
Cathepsin G	90	0·50	32·70 (81%)
Control	90	—	1·0 (2%)

Incubating cathepsin D (EC 3.4.23.5) with GBM at pH 3·4 showed that GBM collagen was not solubilized by cathepsin D.

TABLE 2. *Neutral protease activity of human polymorphonuclear leucocytes*

Results show the mean of two determinations on two healthy donors; 250 ml of blood was taken. Substrates: Z-Ala-2-ONap, *N*-benzyloxycarbonyl-L-alanine 2-naphthyl ester; Bz-DL-Phe-2-ONap, *N*-benzoyl-DL-phenylalanine 2-naphthylester.

Enzyme	Substrate	Activity (units/10^9 leucocytes)
Total neutral proteinase activity	Azocasein	21·4
Elastase	Z-Ala-2-ONap	3·5
Cathepsin G	Bz-DL-Phe-2-ONap	8·1

Discussion

Our results make it clear that human lysosomal elastase and cathepsin G have the ability to degrade human glomerular basement membrane (GBM) *in vitro* at neutral pH. The exact nature of the digestion products was not established, nor did we examine in detail the insoluble material left after digestion. However, since substantially all the hydroxyproline present in the GBM became soluble

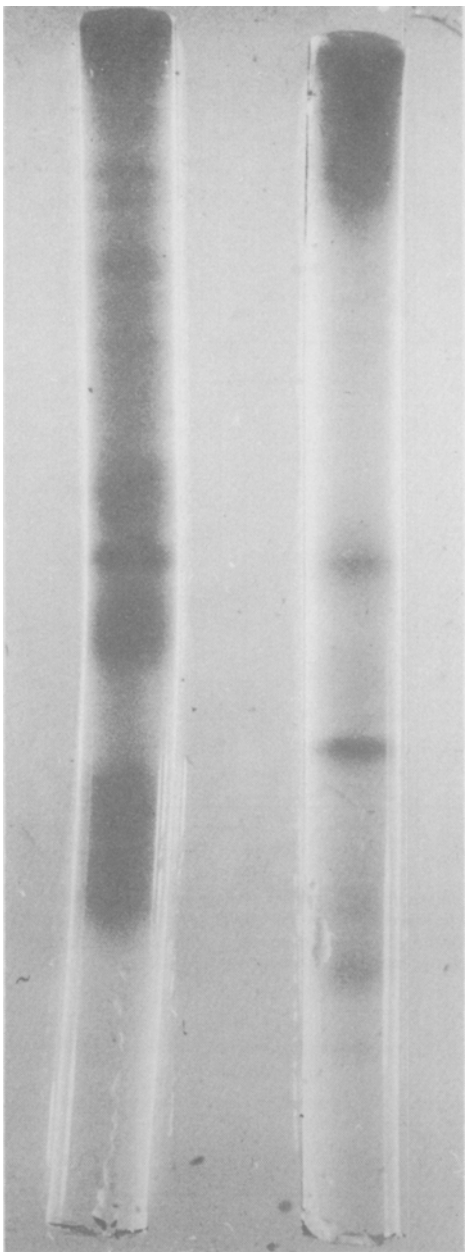

FIG. 3. Electrophoresis in SDS-polyacrylamide gels of human GBM and of fraction 1 isolated by gel chromatography of the products from incubation with neutrophil cathepsin G.

over the incubation period the released products are tentatively identified as collagen or collagen-like proteins. This conclusion is supported by previous studies, which show that a highly specialized collagen (type IV) is present in basement membrane (Kefalides, 1973). Our results also imply that elastase and cathepsin G degrade the GBM in a

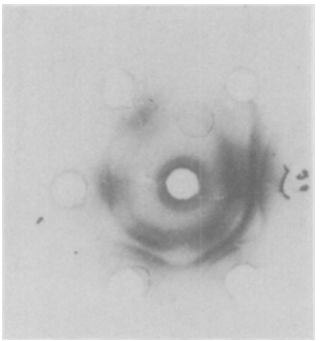

FIG. 4. Immunodiffusion analysis of the soluble proteins released by elastase digestion of GBM. Anti-(human GBM) was placed in the central well and doubling dilutions of the soluble protein in the outer wells.

similar manner. A number of proteolytic enzymes are capable of solubilizing basement-membrane collagen and studies by Rubin, Drake, Davison, Pfahl, Speakman & Schmitt (1965), Drake, Davison, Bump & Schmitt (1966), Kefalides (1969) and Chung, Rhodes & Miller (1976) suggest that these enzymes act by hydrolysing a non-collagen component present in the membrane. Elastase and cathepsin may act in a similar way.

Recent studies have shown that both elastase and cathepsin G act on cartilage as well as GBM in a similar manner to bring about extensive release of proteoglycan and solubilization of collagen, and also that the residual collagen has become degraded in some way (Barrett & Starkey, 1977). The same workers also demonstrated that elastase and to a lesser degree cathepsin G degraded acid-soluble skin collagen. Thus in addition to the type IV collagen of GBM, leucocyte neutral proteinases degrade collagens of types I and II.

It is noteworthy that the protein(s) released by the neutral proteases in our experiments were excluded from Sephadex G-100, although upon acrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate and mercaptoethanol they were resolved into several fractions well within the exclusion limits for this grade of Sephadex. These findings are consistent with other work in which products of GBM degradation have been isolated by gel chromatography. Thus Kefalides (1969) observed that limited digestion of GBM with pronase at 18°C resulted in the solubilization of a fraction that emerged as a single homogeneous peak on Sephadex G-100. Upon electrophoresis this peak resolved into several components resembling interstitial collagen and smaller fragments of GBM collagen. Marquardt, Wilson & Dixon (1973a) showed that analysis of chaotropically

solubilized basement-membrane proteins revealed three prominent bands and up to 19 other less-intense bands, with apparent molecular weights ranging from 28 000 to 94 000. Chung *et al.* (1976) have reported that after pepsin digestion of several human tissues a high-molecular-weight type IV collagen was released, which after reduction and rechromatography on the same column was recovered as collagen-like subunits with apparent molecular weight of 50 000.

Our immunochemical analysis of the solubilized membrane also demonstrated that digestion of GBM with elastase and cathepsin G released a complex mixture of proteins, at least four distinct precipitin lines being detected by rabbit anti-(human GBM) antiserum. Several reports have suggested that a number of major antigenic components are present in GBM (Kefalides, 1973; Marquardt *et al.*, 1973a,b). Studies to date have identified antigenic determinants in the collagen component as well as in low- and high-molecular-weight glycoproteins of GBM.

Two main types of granules, azurophil and specific, have been identified morphologically and biochemically in human polymorphonuclear leucocytes (Bainton, Ullyot & Farquahar, 1971; Bretz & Baggiolini, 1974; Dewald, Rindler-Ludwig, Bretz & Baggiolini, 1975). Elastase and cathepsin G are localized together with other lysosomal hydrolases in the azurophil granules (Dewald *et al.*, 1975). In contrast, neutral collagenase, the third major proteinase of human leucocyte, is exclusively found in the specific granules (Murphy, Reynolds, Bretz & Baggiolini, 1977). This finding may explain the higher rate of release for elastase and cathepsin G and other hydrolases over that of collagenase from phagocytosing polymorphonuclear leucocytes (Oransky, Perper & Schroder, 1973). This raises the question of whether leucocyte collagenase is involved in GBM breakdown. There are numerous reports which indicate that collagenases of bacterial origin digest and solubilize significant amounts of GBM (Spiro, 1967; Kefalides, 1973; Mathieu & Winand, 1973), but so far there is no evidence to indicate that leucocyte collagenase digests human GBM.

We found that cathepsin B was the most effective of the four tissue proteinases studied. Cathepsin B was not, however, detectable in human polymorphonuclear leucocytes, but this thiol-dependent enzyme is present in human kidney (M. Davies, unpublished results). In contrast, cathepsin D, a carboxyl proteinase also found in lysosomes, had no effect. These results are in agreement with reports which show that cathepsin B, but not cathepsin D, degrades native collagen at acid pH (Burleigh *et al.*, 1974; Etherington, 1974). At present it is uncertain what enzymes are responsible for the breakdown of GBM components under normal conditions. From our results it would appear possible that kidney lysosomes through the action of cathepsin B may play a significant role.

The idea that neutral proteinases of leucocytes may induce damage to the GBM is supported by numerous studies that clearly show these enzymes are released when leucocytes come into contact with non-phagocytosable particles, such as immune complexes or aggregated IgG (for review see Ignarro, 1975). In glomerulonephritis deposits of immunoglobulins and complement factors are found along the basement membrane of diseased glomeruli. Further, light- and electron-microscopy show that the polymorphonuclear leucocytes can accumulate in such glomeruli. Studies of renal biopsies both in experimental and human glomerulonephritis clearly indicate that the leucocytes gain intimate contact with the basement membrane by positioning themselves between this structure and the endothelial cells. Our degradation experiments were carried out with the neutral proteinase activity equivalent to 50 ml of whole blood. There are no definitive data on the leucocyte content of diseased kidneys, but our unpublished results would suggest that it is not unusual for more than five polymorphonuclear leucocytes to be present per glomerulus in thin tissue sections, in proliferative glomerulonephritis. Therefore the concentration of purified enzyme used *in vitro* would not seem excessive *in vivo*. We have found that the urine of patients with rapidly progressive glomerulonephritis contains both an increased amount of GBM fragments, and also a neutral proteinase having many of the properties of leucocyte elastase (Sanders *et al.*, 1976). There is thus a great deal of evidence indicating that our results obtained *in vitro* with leucocyte neutral proteases are directly relevant to the pathological process of GBM degradation by leucocytes *in vivo*.

References

BAINTON, D.F., ULLYOT, J.L. & FARQUAHAR, M.G. (1971) The development of neutrophilic polymorphonuclear leucocytes in human bone marrow. Origin and content of azurophil and specific granules. *Journal of Experimental Medicine*, **134**, 907–934.

BARRETT, A.J. (1973) Human cathepsin B1. Purification and some properties of the enzyme. *Biochemical Journal*, **131**, 809–822.

BARRETT, A.J. (1976) An improved color reagent for use in Barrett's assay of cathepsin B. *Analytical Biochemistry*, **76**, 374–376.

BARRETT, A.J. & STARKEY, P.M. (1977) The possible role of leucocyte proteinase in the tissue damage of rheumatoid arthritis. In: *Rheumatoid Arthritis*, pp. 211–221. Ed. Gordon, J. L. & Hazleman, B. L. Elsevier/North Holland, Amsterdam.

BAUGH, R.J. & TRAVIS, J. (1976) Human leucocyte granule elastase. Rapid isolation and characterisation. *Biochemistry*, **15**, 836–841.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scandinavian Journal of Clinical and Laboratory Investigation*, **21** (Suppl. 97), 77–89.

BRETZ, U. & BAGGIOLINI, M. (1974) Biochemical and morphological characterisation of azurophil and specific granules of human polymorphonuclear leucocytes. *Journal of Cell Biology*, **63**, 251–269.

BURLEIGH, M.C., BARRETT, A.J. & LAZARUS, G.S. (1974) Cathepsin B1. A lysosomal enzyme that degrades native collagen. *Biochemical Journal*, **137**, 387–398.

CHUNG, E., RHODES, R.K. & MILLER, E.J. (1976) Isolation of three collagenous components of probable basement membrane origin from several tissues. *Biochemical and Biophysical Research Communications*, **71**, 1167–1174.

DEWALD, B.R., RINDLER-LUDWIG, U., BRETZ, U. & BAGGIOLINI, M. (1975) Subcellular localisation and heterogeneity of neutral proteases in neutrophilic polymorphonuclear leucocytes. *Journal of Experimental Medicine*, **141**, 709–723.

DIXON, F.J. (1968) The pathogenesis of glomerulonephritis: a review. *American Journal of Medicine*, **44**, 493–498.

DRAKE, M.P., DAVISON, P.F., BUMP, S. & SCHMITT, F.D. (1966) Action of proteolytic enzymes on tropocollagen and insoluble collagen. *Biochemistry*, **5**, 301–312.

ETHERINGTON, D.J. (1974) The purification of bovine cathepsin B1 and its mode of action on bovine collagens. *Biochemical Journal*, **137**, 547–557.

IGNARRO, L.J. (1975) Regulation of lysosomal enzyme release by prostaglandins, autonomic neurohormones and cyclic nucleotides. In: *Lysosomes in Biology and Pathology*, **4**, 481–523. North Holland, Amsterdam.

KEFALIDES, N.A. (1969) Characterisation of the collagen from lens capsule and glomerular basement membranes. In: *Diabetes: Proceeding of the Sixth Congress of the International Diabetes Federation*, pp. 307–321.

KEFALIDES, N.A. (1973) Structure and biosynthesis of basement membrane. *International Reviews of Connective Tissue Research*, **6**, 63–104.

KRAKHOWER, C.A. & GREENSPON, S.A. (1951) Localisation of the nephrotoxic antigen within the isolated renal glomerulus. *American Medical Association Archives of Pathology*, **51**, 629–639.

MARQUARDT, H., WILSON, C.B. & DIXON, F.J. (1973a) Human glomerular basement membrane. Selective solubilisation with chaotropes and chemical immunologic characterisation of its compound. *Biochemistry*, **12**, 3260–3266.

MARQUARDT, H., WILSON, C.B. & DIXON, F.J. (1973b) Isolation and immunological characterisation of human glomerular basement membrane antigens. *Kidney International*, **3**, 57–65.

MATHIEU, P.M. & WINAND, R.J. (1973) Carbohydrate and amino-acid composition of human glomerular basement fractions purified by affinity chromatography. *European Journal of Biochemistry*, **37**, 157–163.

MURPHY, G., REYNOLDS, G.J., BRETZ, U. & BAGGIOLINI, M. (1977) Collagenase is a component of the specific granules of human neutrophil leucocytes. *Biochemical Journal*, **162**, 195–197.

ORANSKY, A.L., PERPER, R.J. & SCHRODER, H.C. (1973) Phagocytic release and activation of human leucocyte procollagenase. *Nature (London)*, **246**, 417–419.

RUBIN, A.L., DRAKE, M.P., DAVISON, P.F., PFAHL, D., SPEAKMAN, P.T. & SCHMITT, F.O. (1965) Effects of pepsin treatment on the interaction properties of tropocollagen macromolecules. *Biochemistry*, **4**, 181–190.

SANDERS, E., COLES, G.A. & DAVIES, M. (1976) Polymorphonuclear leucocyte enzyme activity in human glomerulonephritis. *Proceedings European Dialysis and Transplantation Association*, **13**, 541–545.

SPIRO, R.G. (1967) Studies on the renal glomerular basement membrane. *Journal of Biological Chemistry*, **242**, 1915–1922.

STARKEY, P.M. & BARRETT, A.J. (1976) Neutral proteinases of human spleen. Purification and criteria for homogeneity of elastase and cathepsin G. *Biochemical Journal*, **155**, 255–263.