Immunology 1982 **46** 737

The degradation of serum amyloid A protein by activated polymorphonuclear leucocytes: participation of granulocytic elastase

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Accepted for publication 26 January 1982

Summary. To determine the role of inflammation in amyloidogenesis, we have studied the degradation of human serum amyloid A (SAA) protein by purified preparations of human blood polymorphonuclear leucocytes (PMN) and monocytes. When both PMN and monocytes were incubated in SAA-containing medium, the concentration of SAA as measured by a competitive anti-AA radioimmunoassay decreased over time. The rate of decrease of SAA was similar for both monocytes and PMN and there were no differences between four patients with amyloidosis and three normal controls. Resting PMN from normal volunteers were able to degrade SAA to smaller acid-soluble peptides within 16 hr while zymosan-activated PMN produced significant degradation within 1 hr (31%–50%). The supernatants from zymosan-treated PMN also caused marked SAA degradation within 1 hr.

The following enzyme inhibitors were able to prevent degradation of SAA by PMN supernatants; phenylmethylsulphonyl fluoride, a serine esterase inhibitor; α_1 anti-trypsin and soybean trypsin inhibitor; and acetyl-ala-ala-pro-val-chloromethyl ketone, an elastase inhibitor. The ability of a neutral lysosomal enzyme to degrade SAA was further confirmed by showing that purified PMN elastase significantly degraded ^{125}I-SAA.

We conclude that PMN contain one or more lysosomal enzymes capable of degrading SAA, an apoprotein of HDL_3 serum lipoproteins. Alteration in SAA proteolysis by activated PMN may contribute to the deposition of amyloid fibrils in the tissues of patients with chronic inflammatory disease.

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0019-2805/82/0800-0737$02.00

INTRODUCTION

The pathogenesis of secondary amyloidosis is unknown and no consistent humoral or cellular immunological abnormalities have been recognized. Chemical studies have shown that amyloid fibrils are not homogeneous. Whereas the fibrils in primary amyloid are derived from immunoglobulin light chains, the major constituent of secondary amyloid deposits is a protein of unique amino acid sequence called amyloid A (AA) protein. Using an antibody produced against AA protein, an antigenically related 12,500 mol. wt component, serum amyloid A (SAA), has been detected in human serum. SAA is an acute phase reactant, whose concentration increases with

inflammation: SAA has also been recognized as an apoprotein of the HDL_3 fraction of lipoproteins. It is generally assumed that SAA is cleaved to AA in tissues where amyloid fibrils are deposited and there is evidence that SAA may be broken down by enzymes of monocytic origin (Lavie, Zucker-Franklin & Franklin, 1980b).

The role of human blood polymorphonuclear leucocytes (PMN) in the pathogenesis of amyloid disease needs further clarification. It was originally suggested that neutrophils are capable of synthesizing SAA (Rosenthal & Sullivan, 1978) but recent studies have clearly shown that the major site of SAA synthesis is the hepatocyte (Benson & Kleiner, 1980; Selinger, McAdam, Kaplan, Sipe, Vogel & Rosenstreich, 1980). We now show for the first time that the primary function of PMN in amyloidogenesis may be degradation of SAA protein rather than synthesis. Unlike monocytes, where the maximal degradation of SAA appears to reside in cell-membrane-associated enzymes, one of the principal PMN proteases involved in this reaction appears to be an elastase that is released into the supernatants of zymosan-activated human granulocytes. Our studies also show that SAA degradation may be blocked by several neutral protease inhibitors including N-acetyl-ala-ala-pro-val-chlormethyl ketone, a specific anti-elastase agent.

MATERIALS AND METHODS

Reagents

Zymosan particles (Sigma, St. Louis, Mo.) were suspended in boiling water for 5 min and washed by centrifugation with 0·15 M NaCl. The particles were resuspended at a concentration of 10 mg/ml in serum obtained immediately before use and incubated for 30 min at 37°. The serum-treated zymosan particles were washed twice and then resuspended to 1 mg/ml in RPMI medium. The following protease inhibitors were obtained from Sigma: N-a-p-tosyl-L lysine chloromethyl ketone (TLCK); L-l-tosylamide-2-phenyl-chloromethyl ketone (TPCK); phenylmethyl sulphonyl fluoride (PMSF); $\alpha_1$anti-trypsin (α_1AT); soybean trypsin inhibitor; pepstatin; antipain and leupeptin. N-acetyl-ala-ala-pro-val-chloromethyl ketone was kindly donated by Dr Powers of the Georgia Institute of Technology and neutral peptide generating protease (NPGP) was obtained as a gift from Dr Bruce Wintroub of Harvard Medical School. Elastase was purified by the method of Baugh & Travis (1976) and provided by Drs Phillip Stone and Carl Franzblau of Boston University School of Medicine.

Preparation of SAA

SAA was isolated from serum of patients with acute infection and high SAA levels. Serum was acidified with 6 N HCl and chromatographed on a Sephadex G-75 column (2·5 × 95cm) in 5% formic acid. The SAA peak eluted shortly after the void volume peak and was rechromatographed for purification on the same column. The purified SAA gave a single band on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) at approximately 12,500 mol. wt, but was not defined further.

Isolation of leucocyte subpopulations

Peripheral blood PMN and monocytes were isolated from patients with primary and secondary amyloidosis, acute infection and controls. Simultaneous serum was obtained and frozen at −70°. Thirty millilitres of blood (diluted 1:3 with 0·15 M NaCl and containing heparin as anticoagulant) were layered onto cushions of Ficoll-Hypaque (density = 1·077) and centrifuged at 400 *g* for 30 min at 22° (Bøyum, 1968). Mononuclear cells at the plasma: Ficoll-Hypaque interface were washed twice with cold Hanks's balanced salts solution (HBSS) and resuspended at 2×10^6 ml in RPMI-1640 medium with 25 mm Hepes buffer and penicillin-streptomycin, supplemented with 10% foetal calf serum. Monocytes were separated from the mononuclear cell interface by adherence to Falcon 3002 culture dishes for 1 hr at 37°. After washing to remove non-adherent cells, the adherent cells were subsequently incubated at 4° for at least 1 hr to facilitate their removal with a rubber policeman. These adherent cells were greater than 95% monocytes as judged by latex ingestion and morphology. PMN were isolated from the Ficoll-Hypaque pellet. The pellet was mixed with an equal volume of 3 gm% Dextran 500T (Pharmacia, Piscataway, N.J.) in normal saline. After 30 min at room temperature two layers had separated. The top layer which contained the PMN was removed and the cells pelleted by centrifugation. The resulting neutrophil rich pellet was shocked for 20 sec in ice cold 0·2% saline followed by an equal volume of 1·6% saline to remove erythrocytes. The resulting PMN were greater than 99% pure as judged by morphology, and resuspended in RPMI 1640 with 5% horse and 5% foetal calf serum.

Measurement of SAA

SAA was determined by one of two methods: (i) a competitive binding radioimmunoassay using anti-AA antiserum as described previously (Benson, Scheinberg, Shirahama, Cathcart & Skinner, 1977) and (ii) the direct measurement of acid precipitable ^{125}I-SAA. In the latter technique, non-degraded protein is precipitated by 10% trichloroacetic acid (TCA) on glass fibre filters, while protein degraded to smaller peptides is acid soluble. The difference between the total count of SAA added and the acid-precipitable counts is taken as a measure of SAA degradation. SAA was iodinated by a modification of the method of Greenwood, Hunter & Glover (1963) using chloramine T. Labelled protein was separated from excess iodine on a bovine serum albumin (BSA)-coated Sephadex G-25 column. The fractions of iodinated SAA protein with the greatest numbers of counts were used. The usual specific activity of the ^{125}I-SAA was approximately 10^6 c.p.m./ng SAA.

Experimental design

In initial experiments the ability of PMN to degrade SAA was compared with the known ability of monocytes to degrade SAA. Monocytes and PMN were resuspended at $1–10 \times 10^6$/ml in 4·0 ml of RPMI with 0·2% BSA (RPMI-BSA) to which SAA was added at 7 ng/ml. The cell suspensions were incubated at 37° and harvested by centrifugation at 5 min (to correct for non-specific absorption) and at 1, 4 and 14 hr, respectively. The amount of SAA remaining in the supernatant at each point in time was measured by anti-AA radioimmunoassay.

Since disappearance of SAA from the medium could represent ingestion without degradation to smaller peptides, the experiments were repeated using acid precipitable counts. In this method intact proteins are acid precipitable while degraded proteins are acid soluble peptides. PMN were resuspended at 2×10^6/ml in RPMI-BSA containing 10 μg/ml ^{125}I-SAA. In some experiments the media also contained 1 mg/ml opsonized zymosan which was prepared immediately before use. The mixture was then assayed for acid-precipitable SAA at 1, 2 and 14 hr after the addition of equal volumes of 10% cold TCA. The resulting precipitate was then harvested on a Whatman glass fibre filter and counted in a gamma counter. Results are expressed as the mean of triplicate determinations. Suitable controls using SAA without cells and SAA with zymosan were run in parallel. In similar fashion 0·5 ml of the supernatant of 5×10^6 PMN activated with 1 mg/ml zymosan or 1 μg/ml phorbol myristate acetate (PMA) were added to 0·5 ml of ^{125}I-SAA and incubated at 37° in 5% CO_2. The reaction was harvested as above. For kinetic experiments the reaction was allowed to proceed for various time intervals.

The enzymes involved were characterized further by use of various enzyme inhibitors. Supernatant (0·5 ml) of 10^7 zymosan-activated PMN/ml was incubated with 0·5 ml of RPMI-BSA containing ^{125}I-SAA, and 0·1 ml of inhibitors at the following concentrations: PMSF 10^{-3}M, 10^{-4}M, 10^{-5}M; α_1AT 100 μg/ml, 50 μg/ml, 10 μg/ml, 1 μg/ml; TPCK, and TLCK at 5×10^{-4}M; soybean trypsin inhibitor at 100 μg/ml; antipain and leupeptin at 5×10^{-4}M; pepstatin at 5×10^{-4}M and N-acetyl-ala-ala-pro-val CH_2CL at 60 μg and 120 μg/ml. Finally, the degradative activity of purified NPGP and elastase was assayed by incubating 0·5 ml of purified enzyme with 0·5 ml of ^{125}I-SAA for 1 hr. NPGP was assayed at final concentration of 0·312, 0·625, 1·25 and 2·5 μg/ml. Elastase was assayed at 5 μg, 20 μg and 40 μg/ml. The ratios by weight of enzyme to substrate used were as follows: NPGP to SAA 1:2; 1:4, 1:8 and 1:16; elastase to SAA 1:1·25, 1:2·5; and 1:10, respectively.

RESULTS

Degradation of SAA by PMN and monocytes

The degradation of SAA by PMN and monocytes was initially determined in four patients with amyloidosis and three healthy laboratory personnel using the anti-AA radioimmunoassay. Several cell concentrations were studied and maximal viability of cells and SAA degradation occurred at 2×10^6/ml (Table 1). Both PMN and monocytes caused disappearance of SAA from the medium in all individuals. In some individuals the SAA disappeared rapidly in the first hour while in others it disappeared more slowly over the entire experimental duration of 14 hr. The rate of disappearance of SAA from the medium in the presence of PMN roughly paralleled the rate of disappearance of SAA in the presence of monocytes in all individuals studied. Using trypan blue, the viability of the PMN was greater than 90% until 8 hr when it slowly began to decrease to less than 80% viability at 14 hr. Because of the decrease in viability and the possible 'leakage' into the medium of granule associated enzymes, the experiment was terminated at 14 hr. In three donors where no SAA was added, no

Table 1. Degradation of SAA by PMN and monocytes as measured by radioimmunoassay

Diagnosis	SAA concentration PMN (ng/ml)				SAA concentration monocytes (ng/ml)			
	0 Hr	1 Hr	4 Hr	14 Hr	0 Hr	1 Hr	4 Hr	14 Hr
Normal donor	7·0	5·1	5·0	1·0	7·0	5·9	5·0	3·1
Normal donor	7·0	2·8	2·8	1·7	7·0	3·7	3·6	3·0
Normal donor	7·0	4·5	3·8	3·8	7·0	4·7	4·0	3·6
2° Amyloid	7·0	2·7	2·0	1·4	7·0	2·9	2·6	1·3
2° Amyloid	7·0	3·1	2·8	2·2	7·0	3·3	2·5	1·6
2° Amyloid	7·0	3·4	3·3	1·2		Not tested		
1° Amyloid	7·0	3·9	4·0	1·4		Not tested		

appreciable synthesis of SAA by PMN was observed during the 14 hr experiment.

The degradation of SAA was then measured using the method of acid-precipitable counts. Unstimulated PMN in medium were found capable of decreasing the amount of acid precipitable SAA (15% in 2 hr; 49% in 14 hr). Since secondary amyloidosis is associated with chronic inflammation or infection, we also chose to study the degradative ability of PMN after stimulation by opsonized particles. PMN that were stimulated with serum-treated zymosan particles were capable of even greater degradation than non-activated PMN (33% versus 8% in 1 hr; Table 2). To exclude the role of non-specific binding to PMN, ^{125}I was added to azide treated PMN. At 1 hr no significant decrease in acid precipitable SAA was seen (data not shown).

Table 2. ^{125}I-SAA degradation by normal PMN after 1 hr

	Mean degradation (%)
^{125}I-SAA control	0
^{125}I-SAA + zymosan	2
^{125}I-SAA + PMN ($n=2$)	8
^{125}I-SAA + zymosan PMN ($n=2$)	33

Degradation of SAA by PMN supernatants

To differentiate between intracellular and extracellular degradation to acid soluble peptides, supernatants obtained from unstimulated PMN or PMN stimulated for 60 min with opsonized zymosan 1 mg/ml, respectively were incubated with labelled SAA. Degradation was measured by acid-precipitable counts after 1 hr (Table 3). No degradation of SAA was observed by the supernatants of unstimulated PMN but the supernatants of zymosan-treated PMN significantly degraded SAA (mean 56·9%). The supernatants of PMN stimulated by phorbolmyristate acetate did not degrade SAA significantly more than unstimulated PMN.

The degradation of SAA by supernatants from zymosan-activated PMN suggested the use of the supernatants in a cell-free system to characterize further the degradation. The kinetics of SAA degradation are rapid (Table 4) with significant breakdown

Table 3. ^{125}I-SAA degradation by normal PMN supernatants after 1 hr

	Mean c.p.m.	Mean degradation (%)
^{125}I-SAA Control	55,054 ± 4320	0
Supernatant of zymosan alone	54,278 ± 2321	1·4
Supernatant of PMA alone	53,765 ± 3012	2·5
Supernatant of PMNs in media ($n=5$)	51,946 ± 3941	5·6
Supernatant of zymosan + PMNs ($n=5$)	23,733 ± 1660	56·9
Supernatant of PMA + PMNs ($n=5$)	51,396 ± 1691	6·6

Table 4. Kinetics of SAA degradation by normal PMN supernatants

Time (min)	Supernatant no. 1 (c.p.m.)	Supernatant no. 2 (c.p.m.)	Mean SAA degradation (%)
0	25,540±677	25,540±677	
15	19,839±592	25,076±801	12
30	19,117±709	20,951±104	22
60	12,173±405	19,160±662	39
120	9117±1331	12,186±138	58

Table 5. Effect of protease inhibitors on SAA degradation by normal PMN supernatants

	Case no. 1 c.p.m.	Case no. 2 c.p.m.	Mean degradation (%) of control	Mean effect (%) of inhibitor
(a) ^{125}I-SAA control	28,416±1076	24,120±1736	—	—
PMN supernatant alone	9910±574	7945±1961	66·1	—
With inhibitors:				
PMSF* 10^{-3} M	24,864±862	27,721±3667	11·2	83·1
10^{-4} M	23,468±3379	20,939±3800	15·5	76·9
10^{-5} M	13,331±1198	17,259±1055	40·8	38·4
α_1-antitrypsin 100 μg/ml	25,567±3024	21,620±7148	10·2	84·6
50 μg/ml	25,642±2800	19,013±2668	15·5	76·6
10 μg/ml	11,810±328	7546±1240	63·6	6·1
1 μg/ml	8805±314	8104±2096	67·7	0
(b) ^{125}I-SAA control	27,937±343	27,937±343		
PMN Supernatant alone	16,714±3232	15,469±1108	42·4	—
With inhibitors				
TPCK† 5×10^{-4} M	18,535±1220	18,219±2149	34·2	19·3
TLCK‡ 5×10^{-4} M	17,723±1103	17,754±1826	36·5	13·9
Soybean trypsin 100 μg/ml	27,592±332	26,917±1872	2·5	94·1
Antipain 5×10^{-4} M	Not tested	17,166	38·6	9·0
Leupeptin 5×10^{-4} M	Not tested	15,619	44·1	0
(c) ^{125}I-SAA control	92,537±911	92,537±911	—	—
PMN Supernatant alone	36,938±5020	38,915±3510	59·0	—
With inhibitors:				
Pepstatin 5×10^{-4} M	69,217±4498	64,693±140	27·6	53·2
5×10^{-5} M	45,020±2747	39,505±2906	54·3	7·9
Ac-ala-ala-pro-val-Ch_2Cl 60 μg/ml	80,274±1897	65,735±1400	21·1	64·2
120 μg/ml	81,016±1410	82,953±2517	11·4	80·7

* PMSF, phenylmethyl sulphonyl fluoride.
† TPCK, L-tosylamide phenylethyl chloromethyl ketone.
‡ TLCK, N-a-p-tosyl-L-lysine chloromethyl ketone.

(22%) occurring in 30 min, and greater than 50% in 120 min. The activity of the supernatant was also titrated. Significant degradation occurred at dilution of 25% or less. There was no pH dependence of the degradation between pH 6·5 and 8·6 when the reaction was performed in Tris buffers of varying pH.

Use of enzyme inhibitors to characterize the reaction

To define further the enzymes involved in the degradation of SAA, various inhibitors were added to the reaction mixture of supernatant and labelled SAA. PMSF, a serine esterase inhibitor, as well as two non-specific protease inhibitors, α_1 anti-trypsin and

Table 6. SAA degradation by neutral peptide generating protease and PMW elastase

	Concentration (μg/ml)	c.p.m.	Degradation (%)
(a) ^{125}I-SAA control (10 μg)		38,001 ± 2373	—
NPGP	2·5	42,161 ± 2274	0
NPGP	1·25	42,402 ± 309	0
NPGP	0·625	42,752 ± 1307	0
NPGP	0·312	42,356 ± 138	0
(b) ^{125}I-SAA control (100 μg)		92,884 ± 5558	—
PMN elastase	40	42,005 ± 495	54·8
PMN elastase	20	55,129 ± 2511	40·6
PMN elastase	5	69,570 ± 3572	25·1

soybean trypsin inhibitor, gave 70% inhibition at 10^{-4}M (Table 5a). Both TLCK and TPCK that inhibit chymotrypsin and trypsin-like enzymes, blocked degradation to a lesser extent at 10^{-4}M (Table 5b). At 5×10^{-4}M, but not 5×10^{-5}M, the breakdown of SAA was markedly inhibited by pepstatin, an inhibitor of acid proteases such as cathepsin D. Other enzyme inhibitors of microbial origin, antipain and leupeptin, respectively, did not significantly inhibit SAA breakdown but the specific granulocyte elastase inhibitor, N-acetyl-ala-ala-pro-val-chloremethyl ketone gave 81% inhibition at a concentration of 120 μg/ml (Table 5c).

Degradative activity of purified enzymes

Previous studies on monocytes and amyloidosis suggested the involvement of a membrane-bound enzyme. One such newly purified preparation, NPGP, (Coblyn, Austen & Wintraub, 1979) was tested but failed to cleave SAA protein at 0·3–2·5 μg/ml concentrations and enzyme-substrate ratios of 1:2 to 1:16 (Table 6a). Inhibition of SAA degradation by the elastase inhibitors prompted the final experiment in which a purified preparation of PMN elastase provided 25%–55% SAA degradation *in vitro* at enzyme substrate ratios of 1:1 to 1:10 (Table 6b).

DISCUSSION

Investigation of both man and mouse suggests that individuals or inbred murine strains differ in their susceptibility to secondary amyloid disease. The CBA mouse has a marked tendency to develop casein-induced amyloidosis while the A mouse is more resistant. This resistance has been correlated with a single recessive gene (Wohlgethan & Cathcart, 1979). Susceptibility to secondary amyloidosis may also be related to excessive production of SAA or to abnormal clearance (processing to AA and smaller peptides). All humans studied produced SAA in large amounts following infections such as pneumonia and osteomyelitis, yet only a small percentage of patients with chronic inflammatory disease develop amyloidosis.

It has been suggested that proteolytic enzymes play a key role in the pathogenesis of primary amyloidosis (Linke, Zucker-Franklin & Franklin, 1973). Epstein, Tan & Wood (1974) have demonstrated formation of amyloid fibrils *in vitro* by action of human kidney lysosomal enzymes on Bence Jones proteins. Lavie, Zucker-Franklin & Franklin (1978) showed that peripheral blood monocytes degrade SAA protein along three pathways. Of twenty normal subjects, eight were able to cleave SAA completely with no detectable intermediates, eight transiently produced AA-like polypeptides and four yielded a persistent AA-like intermediate protein. Cells from patients with both primary and secondary amyloidosis fell into the second group. The responsible enzymes were identified as membrane-associated proteases, although other investigators have recently described similar protease activity in the fluid phase as well (Skogen, Thorsteinsson & Natvig, 1980).

In experimental models, PMN may play an equivalent or more important role. The final amyloid deposition phase is markedly accelerated by acute inflammation (Kisilevsky, Axelrad, Corbett, Brunet & Scott, 1977) and leucocytosis (Kedar & Ravid, 1980) and many strains of mice, including the A strain fail to show the monocyte abnormality associated with the human form of the disease (Lavie, Franklin & Zucker-

Franklin, 1980a). The aim of the present study was to determine if peripheral blood PMN can participate in the clearance by degradation of SAA proteins to smaller peptides.

In all individuals studied unstimulated PMN reduced the concentration of AA-like proteins over time. The rate of decrease varied but there was no consistent difference between patients with amyloidosis and controls. In each case the rates of decrease of SAA concentration by both monocytes and neutrophils were similar. To prove that disappearance of SAA protein from the cultures was due to degradation and not simply ingestion by PMN, the concentration of ^{125}I-SAA was determined in experiments using acid precipitation techniques. PMN were incubated in medium containing the radiolabelled substrate and the total acid-precipitable counts were measured at varying intervals. Unstimulated resting whole PMN degraded SAA to acid soluble peptides but PMN activated by ingestion of opsonized zymosan particles induced even greater degradation (50% or greater in 1 hr). Although it is possible that SAA was broken down by membrane-associated enzymes or by intracellular release of enzymes into a phagolysosome, the rapid appearance of protease activity in the supernatants of zymosan-activated PMN strongly suggests the operative mechanism to be 'regurgitation during feeding' (Weissman, Dukor & Zurier, 1971). This activity has been identified in two fractions, the azurophilic or primary granule and the specific granule (Baggiolini, Bretz, Dewald & Feigenson, 1978). Since phorbolmyristate acetate at low concentrations is thought to release only the contents of specific granules (Wright, Bralove & Gallin, 1977) the negative results using this reagent indicated that most of the enzyme activity was in the primary granule.

When we tried to characterize the proteolytic enzymes that degrade SAA protein using a number of protease inhibitors, the most effective were N-acetyl-ala-ala-pro-val-chloromethyl ketone, a specific inhibitor of PMN elastase (Powers, Gupton, Harley, Nishino & Whitley, 1977); PMSF, a neutral serine esterase inhibitor; and the α_1 soybean trypsin inhibitors, respectively. Pepstatin, a specific acid hydrolase inhibitor (Aoyagi & Umezawa, 1975) partially blocked the breakdown of SAA protein but other protease inhibitors of microbial origin, leupeptin and antipain were not effective at 10^{-4} concentration. TPCK and TLCK, potent inhibitors of trypsin and chymotrypsin-like enzyme also failed to prevent SAA degradation.

In the final series of experiments, purified PMN elastase was used to cleave SAA protein directly, whereas negative results were obtained using another recently isolated granulocyte fraction, the neutral peptide generating protease or Cathepsin G (Wintroub, personal communication).

Taken as a whole, these findings have implications regarding the pathogenesis of secondary amyloidosis. Human PMN appear to contain one or more proteolytic enzymes that may cleave SAA protein to peptides of smaller size. It is not known if these peptides resemble AA and there may be some difficulty in directly relating our results to different SAA types since several laboratories have identified heterogeneity of SAA in plasma (Bausserman, Herbert & McAdam, 1980). Unlike human monocytes, PMN exert most of their proteolytic activity in a lysosomal fraction rather than on the cell membrane. Our studies indicate that one human lysosomal enzyme in particular, PMN elastase, may play an important and hitherto unrecognized biological role by clearing amyloid precursor proteins and possibly other acute phase reactants from plasma. Only a breakdown in normal tissue homeostasis would result in the deposition of intermediate size AA protein molecules or amyloid fibrils. This would be most likely to occur in certain chronic diseases during episodes of intense inflammation when the balance between neutrophil infiltration and serum SAA levels is markedly disturbed.

ACKNOWLEDGMENTS

The authors thank Dr Robert A. Clark, Boston University Medical Center, for helpful suggestions in the planning of this work. The skilled technical assistance of Ms Linda Burnett and the secretarial assistance of Ms Maura Herlihy are also gratefully acknowledged.

These investigations were supported by grants from the United States Public Health Service, National Institute of Arthritis, Metabolism and Digestive Diseases (AM 26451, AM 04599 and AM 07014) and The Arthritis Foundation.

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