

The Role of The Human Neutrophil in the Inflammatory Reaction

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The neutrophil granulocyte plays a key role in the defence against and the digestion of invading micro-organisms. It has, however, become increasingly clear that the neutrophil should be viewed as a secretory cell. Thus during ingestion and degranulation cytotoxic and other granular agents are deposited not only in phagocytic vacuoles where micro-organisms have been sequestered but also extracellularly. Similarly, signals for degranulation can occur also on a surface (e.g. basement membrane) covered by immune complexes. Even soluble compounds can be signals for degranulation. The result will be extracellular release of granule content on to particulate or soluble substrates leading to tissue damage. In addition neutrophil constituents which are released extracellularly can also give amplification as well as suppression of the inflammatory reaction. A good candidate for all these effects is the group of cationic proteases abundant in human neutrophils and acting at a neutral pH. On the other hand the benefit to the host of these and other neutrophil agents in the defence against micro-organisms is without doubt. The reason for the linkage between defence and tissue injury is that we are dealing with cytotoxic agents. The problem is to see the usefulness, if any, of the extracellular action of neutrophil compounds. If the physiological role of neutrophil proteases is related to intracellular destruction of micro-organisms, extracellular injury can perhaps be viewed as a failure of the protective function of the proteinase inhibitor system.

The purpose of this review is to focus on the biochemical and biological properties of some of the major neutrophil granule constituents. We will concentrate on the cationic neutral proteases, which represent major constituents of such organelles. By virtue of their biochemical and biological characteristics it is at least possible to hypothesize on their role in immunological and allergic conditions.

GRANULE FORMATION IN NEUTROPHILS

Neutrophil granules were first isolated in 1960 (5) and shown to be lysosomal-like. However, human neutrophil granules are particularly rich in substances, e.g. neutral proteases which are usually not associated with lysosomes. Two distinct types of cytoplasmic granules are produced at subsequent stages of granulopoietic maturation (2). Azurophil (primary) granules are formed in promyelocytes and contain peroxidase. Specific (secondary) granules are formed in myelocytes and they are peroxidase negative.

Physical separation of the two major granule populations has been achieved also for human neutrophils by means of centrifugation in density gradients (3, 45). The large protein content is documented by an average density of 1.23 for azurophil and 1.19 for specific granules. The high densities lead to thoughts about the physical state of granule content and questions related to the latency and degranulation. Most granule proteins are characteristi-

cally highly cationic. Neutrophil granules contain anionic glycosaminoglycans (chondroitin sulphate) (37, 38). Therefore it has been suggested that the cationic proteins are neutralized by ionic complex formation with glycosaminoglycans. Proteins are packaged into azurophil granules produced from the proximal or concave part of the Golgi apparatus and specific granules are produced from the distal or convex face of the Golgi (2). The protein part of the chondroitin sulphate proteoglycan newly synthesized on ribosomes is transferred to newly formed granules of the Golgi (36). During this progression chain elongation and sulphation of the polysaccharide occur. Thus the glycosaminoglycans would form a granule matrix of acid nature, which in particular can bind cationic proteins. This model permits extensive packaging and segregation of proteins into neutrophil granules. It would also explain structure-linked latency of granule content which can also be attributed in a more classical fashion to the membrane-like barrier surrounding the granule.

CONTENT OF NEUTROPHIL GRANULES

Our present knowledge of the composition of azurophil and specific granules of human neutrophils is summarized in Table 1. Only major constituents are listed. It is important that the composition varies markedly between the species. Acid proteases like cathepsin D and E have been made responsible for neutrophil-induced tissue injury. However, with human neutrophils neutral proteases like elastase, chymotrypsin-like cationic proteins and collagenases would be the major agents involved in tissue injury and mediation of inflammation. Specific activities of neutral proteases are 10 to 30 times higher in human than rabbit granulocytes (3). Species differences are also emphasized by lack of lysozyme and peroxidase in some species. Rabbit neutrophils contain abundant unique non-enzyme cationic proteins (21, 61).

Determinations of lactoferrin and myeloperoxidase in density gradient fractions identify precisely specific and azurophil granules respectively (3, 45). Studies from several laboratories also agree on the sharp unimodal distribution of elastase and chymotrypsin-like cationic protein, identical to that of myeloperoxidase (3, 34). Consequently, these are part of the azurophil granules. There has been some controversy with regard to the localization of granulocyte collagenase, but it is now clear that an abundant non-specific collagenase resides in azurophil granules (29, 34) while a minor specific collagenase resides in specific granules (22). The specific granules are otherwise less well characterized than azurophils. They are known to contain lysozyme, lactoferrin and a B_{12} -binding protein. Alkaline phosphatase, which is a marker enzyme of specific granules in rabbit neutrophils, is not present in human neutrophil granules (3, 45).

MOLECULAR PROPERTIES OF NEUTROPHIL GRANULE PROTEINS

We shall confine ourselves to the discussion of the neutrophil proteases and a

Table 1
Major constituents of azurophil and specific granules of human neutrophils

Azurophil granules	Specific granules
Acid hydrolases	—
Myeloperoxidase (39.1 ± 9.5)	—
Elastase (27.3 ± 6.6)	—
Chymotrypsin-like cationic protein (12.0 ± 2.2)	—
Non-specific collagenase (41.1 ± 11.5)	Specific collagenase
Lysozyme	Lysozyme
—	Lactoferrin (43.5 ± 12.2)
—	Vit B_{12} -binding protein

The content $\pm$ s.d. per 10^7 neutrophils determined by immunochemical methods (23) is given within parenthesis for some constituents.

Table 2
Biochemical properties of neutrophil proteases

	CCP	Elastase	Collagenase (non-specific)
S°_{20w}	—	2.6	4.5
Mol. wt (SDS-electrophoresis)	25,500–28,500	33,000–36,500	{ 42,000 33,000
pH optimum	7.0–7.5	8.5	8.5
Natural substrates	fibrinogen	elastin	collagen
	proteoglycans	proteoglycans	proteoglycans
	casein	fibrinogen	fibrinogen
	histones	C3, C5	C3
	haemoglobin	IgG	
	C3, C5		
	IgG		

CCP, Chymotrypsin-like cationic protein.

newly defined permeability increasing bactericidal cationic protein. For information on the properties of granule proteins not considered here, the reader should consult other reviews (18). The biochemical properties of the neutrophil proteases are summarized in Table 2.

Chymotrypsin-like cationic protein (Cathepsin G)

In a search for bactericidal cationic proteins similar to those of rabbit and guinea pig (61), we discovered a family of highly cationic proteins in human neutrophil granules (39, 40). Unlike the cationic proteins from rabbit and guinea pig they possess chymotrypsin-like activity (26). We purified four cationic components (40), which appeared homogeneous on SDS-electrophoresis after reduction and alkylation. They ranged in molecular weight from 25,500–28,500. All four components have a nearly identical amino acid composition with a high arginine content. The isoelectric point is above pH 11. The basicity of these proteins is also in part due to masking of carboxylic acids by amide groups. All four components show immunological identity by crossed immunoelectrophoresis or double immunodiffusion employing anti-serum prepared

against individual components. We are obviously dealing with a set of isoenzymes. The constant differences in electrophoretic migration are presumably due to variation in carbohydrate content reflected by the slight variations in molecular weight. Similar cationic esterases were described by others (8, 9, 42, 43). The chymotrypsin-like enzyme purified from human spleen and designated cathepsin G (46), seems to be identical with the neutrophil chymotrypsin-like cationic protein.

This enzyme is a serine protease. In addition to chymotrypsin-specific esters it attacks several natural substrates like fibrinogen, casein, proteoglycans and complement components.

Elastase

The existence of an elastolytic activity of human neutrophils was reported in 1968 (16). It was suspected because of the local haemorrhage produced by dermal injection of human neutrophil granules. Granulocyte elastase has now been isolated and characterized in several laboratories (7, 31, 43). We have purified three isoenzymes (31), which show the reaction of identity on analysis with im-

munodiffusion or crossed electrophoresis. They react like homogeneous proteins when analysed by ultracentrifugation and gel filtration. Electrophoresis of reduced enzyme in SDS suggest molecular weights in the range of 33,000–36,000. The amino acid compositions are virtually identical except for a slight variation in arginine content.

Neutrophil elastase rapidly solubilizes native insoluble elastin with a pH optimum at 8.5. In addition a broad variety of natural substrates are degraded like fibrinogen, proteoglycans and complement components.

Collagenase

Collagenases are produced and secreted by a variety of normal and pathological tissues. Neutrophil collagenases are, however, stored intracellularly in the cytoplasmic granules. Collagenase activity in neutrophil homogenates was reported in 1968 (19). When collagen in solution was used as substrate, cleavage products consisting of 1/4 and 3/4 fragments were obtained typical for mammalian collagenases. Purification resulted in loss of the ability to degrade collagen fibrils.

Our laboratory has succeeded in purification of a neutrophil collagenase (29). This enzyme has now turned out to be different from specific neutrophil collagenase, but it is so far the only neutrophil collagenase which has been purified and characterized in detail. Actually, it is a non-specific collagenase attacking several substrates in addition to collagen (Table 2). On the other hand, it is a major granule constituent in contrast to the specific collagenase, which is a minor component. Therefore it may be of considerable biological importance. Polyacrylamide gel electrophoresis of our purified enzyme revealed one main and one minor component, which were separated by preparative electrophoresis. Both collagenolytic enzymes showed the reaction of immunological identity. The amino acid compositions were almost identical. The molecular weight was estimated at approximately 76,000, but electrophoresis

in SDS revealed two components with molecular weights of 42,000 and 33,000. It is suggested therefore, that each of the isoenzymes is built by two non-identical subunits. In contrast to specific neutrophil collagenase this enzyme is a serine protease as it is inactivated by DFP. The specific collagenase is a constituent of the specific granules of human neutrophils (22). The unspecific but major collagenase is a constituent of azurophil granules (34).

The bactericidal permeability increasing (PI) protein

A cationic protein fraction from rabbit neutrophils is potently bactericidal towards gram-negative bacteria (57). Bacterial killing is accompanied by a discrete increase in microbial envelope permeability. A cationic protein has recently been purified from human neutrophil granules that possess very similar bactericidal and permeability increasing activities (58). Purification by ion exchange and gel chromatography has yielded an apparently single homogeneous protein, as judged from isoelectric focusing and electrophoresis in several systems. This protein has an isoelectric point at pH 9.8. The apparent molecular weight is 58,000 to 60,000. Amino acid analysis revealed a lysine-rich protein. Even if the acidic residues outnumber the basic residues, the apparent isoelectric point of 9.8 suggests that, as in the chymotrypsin-like cationic proteins, acid groups are in the amide form. The purified bactericidal/PI protein has no detectable protease, peroxidase, lysozyme or any other enzyme activity tested. It may therefore be a non-catalytic bactericidal protein. This is in contrast to the partially purified bactericidal/PI fraction from rabbit granulocytes that is associated with phospholipase A₂ (57).

MICROBICIDAL EFFECTS OF NEUTROPHIL PROTEINS

We will limit our discussion here to the granule proteins whose molecular properties have been

Table 3

Microbicidal activity of chymotrypsin-like cationic proteins (CCP) and bactericidal permeability increasing (PI) protein

	CCP	PI/Protein
Susceptible bacteria	gram-positive	gram-negative
pH optimum	neutral	neutral
Heat stability	very high	low
Inhibition by:	5–10 mM Mg ²⁺	5–10 mM Mg ²⁺
	serum protease inhibitors	—
Inhibits bacterial:	oxygen consumption	—
	protein synthesis	—
	DNA —, —	—
	RNA —, —	—
	⁸⁶ Rb ⁺ influx	—
		permeability barrier

outlined above (Table 3). The important oxygen-dependent microbial killing by neutrophils performed by certain oxidases and myeloperoxidase will not be considered. The reader should consult a recent excellent review on the latter subject (1).

Gram-positive bacteria are most susceptible at a neutral pH to the microbicidal effect of chymotrypsin-like cationic protein (24), but the proteolytic and antimicrobial activities appear to be separate functions of these agents; heat inactivation of the proteolytic effect does not affect the microbicidal capacity. One could hypothesize that these proteins have separate domains for catalytic and killing actions. The inverse relationship between microbicidal activity and ionic strength indicates charge interaction between protein and bacterial surface. Absorption to the microbial envelope is required for killing. Complex formation with natural protease inhibitors inhibits microbicidal function. The reason is probably steric hindrance of the interaction between active groups on the protein and the bacterial surface.

The bactericidal mechanism of the chymotrypsin-like proteins is unknown (25). Killing is temporarily accompanied by inhibition of bacterial synthesis of macromolecules

and accumulation of ⁸⁶Rb⁺. The findings are consistent with damage to the cytoplasmic membrane. Such damage, at least initially, is limited, since *S. aureus* preloaded with ⁸⁶Rb⁺ or ¹⁴C-leucine exhibits very little release of either isotope during the first 20 min of incubation. With prolonged incubation, loss of ¹⁴C-leucine is substantial, indicating ultimate structural disintegration.

The bactericidal effect of the permeability increasing (PI) protein (58), is restricted to gram-negative bacteria. The effect occurs without extensive destruction of the microbial envelope. Thus the biosynthesis of macromolecules remains intact despite loss of viability, but killing is accompanied by an immediate increase in permeability of the envelope. It can be measured by inhibition of bacterial protein synthesis in the presence of actinomycin D, to which the gram-negative bacterial envelope is normally impermeable. An increased phospholipid turnover can also be measured. Both these effects are, unlike the bactericidal effect, promptly reversed by the addition of 10 mM Mg²⁺ or Ca²⁺. Endogenous phospholipid degradation does not play an essential role in killing as a phospholipase A-deficient *E. coli* mutant is killed equally well. On the other

hand, the connection between the completely reversible alteration in envelope permeability and irreversible loss of multiplication remains to be determined.

Several killing mechanisms are available to neutrophils for defence against micro-organisms. These are represented both by oxygen-dependent mechanisms and oxygen-independent mechanisms, like those described above. It is not possible to determine exactly which agents are most important for destruction of various micro-organisms under different conditions. It is highly likely, however, that killing is achieved by the concerted action of all the antimicrobial agents available. In addition, we have evidence that elastase and chymotrypsin-like cationic protein, independent of their enzyme activities, in combination with the bactericidal/PI protein produce a far greater bactericidal effect than any of these proteins alone. Moreover, elastase highly potentiated the initial rate of killing by the myeloperoxidase-H₂O₂ system. It is not impossible that the efficient overkill capacity of neutrophils creates some of the undesirable effects during phagocytosis, e.g. damage to the tissues of the host (see below).

PROTECTIVE EFFECT OF NATURAL
PROTEASE INHIBITORS

Plasma contains several protease inhibitors, like α_1 -antitrypsin (AAT), α_2 -

macroglobulin (A_2M) and α_1 -antichymotrypsin (AAC). In addition mucous membranes have a low molecular weight protease inhibitor active against neutrophil proteases (35). The physiological disintegration of neutrophils with release of the neutral proteases may occur preferentially in mucous membranes. Therefore the latter inhibitor may be of great importance both under normal conditions and during inflammation. The plasma protease inhibitors usually have rich access to inflammatory processes because of increased local capillary permeability. Neutrophil proteases released by secretion or cell disintegration are therefore rapidly diluted and inactivated provided that the capacity of the inhibitors does not become saturated. The latter can occur for example in abscess contents. However, neutrophil proteases can be released directly on particulate substrates, e.g. immune complexes on basement membranes. The micro-compartment formed between phagocyte and substrate may contain a very high enzyme content. As plasma inhibitors are perhaps physically excluded, tissue damage and degradation can now occur.

Interaction between plasma protease inhibitors and neutrophil proteases is summarized in Table 4.

Chymotrypsin-like cationic protein is inactivated both by A_2M , AAT and AAC (27, 53). The highest affinity is found for A_2M . Complexes with AAT were labile and enzyme

Table 4
Specificity of major protease inhibitors

	Mol.wt.	Inhibition of neutrophil proteases		
		CCP	Elastase	Non-specific collagenase
α_1 -Antitrypsin	55,000	(+)	+	+
α_1 -Antichymotrypsin	69,000	+	—	—
α_2 -Macroglobulin	725,000	+	+	+
Bronchial mucus inhibitor	10,500	+	+	—

CCP, Chymotrypsin-like cationic protein.

shifted to A_2M when this inhibitor was added. Chymotrypsin-like cationic protein may be particularly destructive to tissues and cells because of its relative resistance to AAT, especially in the presence of other proteases with higher affinity for AAT.

Neutrophil elastase is inactivated by serum because of complex formation with AAT and A_2M (7, 30). The affinity is similar for both inhibitors but AAT is probably the most important elastase inhibitor *in vivo* due to its higher molar concentration. A_2M -complexes injected intravenously in human volunteers (using ^{131}I -labelled enzyme) disappeared with a half-life of 12 min (28). The AAT-complexes on the other hand showed a half-life of 1 h.. They had a tendency to dissociate with transfer of elastase to A_2M . Thus even if AAT is the most important elastase inhibitor A_2M is important for elimination of the protease.

Non-specific neutrophil collagenase is inactivated both by AAT and A_2M (32). However, in contrast to elastase it showed 10

times higher affinity for A_2M than AAT. Transfer of collagenase from AAT to A_2M was observed.

EXTRACELLULAR EFFECTS OF NEUTROPHIL-DERIVED CONSTITUENTS IN THE INFLAMMATORY REACTION

Microbicidal action is assumed to be the primary function of the neutrophil. Evidence indicates, however, that damage to the host can occur in the inflammatory reaction, in particular as an effect of the extracellular neutrophil proteases. The extracellular deposition of granule constituents is known to be brought about by a number of mechanisms, e.g. cell death, regurgitation during phagocytosis, release during migration, secretion, and reversed endocytosis (Fig. 1) (44). Specific granule content is released extracellularly to a higher degree than the content of azurophil granules (20), which suggests differences in the

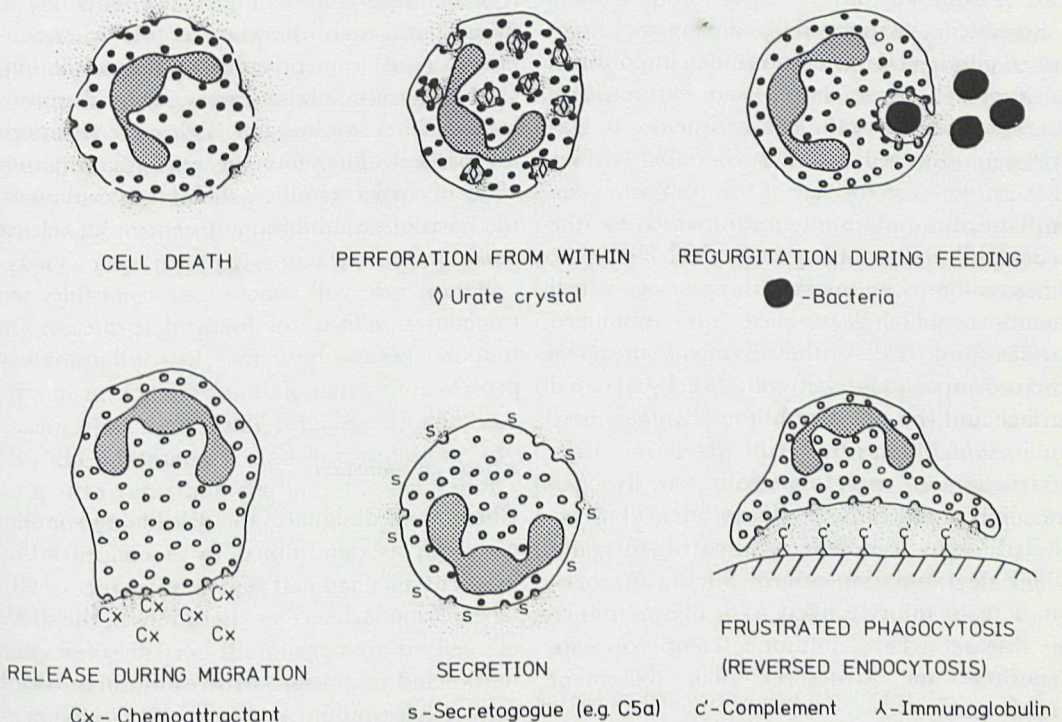


Fig. 1. Mechanisms for extracellular release of neutrophil granule content.

mechanisms governing the release from the two types of granules. Heat-labile serum factors preferentially stimulate release of azurophil granule content (44), while cations like Ca^{++} and Mn^{++} stimulate secretion from specific granule content (44, 51).

In the past, extracellular damage by neutrophils has usually been regarded as an accidental process secondary to lysis of the cell. Lysis can be produced by a variety of toxins and detergents (44). Monosodium urate and silica can gain access to the interior of the cell and cause "perforation from within" with subsequent extracellular deposition of both granule and cytoplasmic constituents. This mechanism is likely to induce the inflammatory reaction to gout.

Another mechanism for tissue injury is regurgitation of granule enzymes to the exterior of the cell during phagocytosis. This is probably a consequence of incomplete closure of the phagosome upon ingestion. Regurgitation of neutral proteases has been convincingly shown to occur during phagocytosis of zymosan (33).

So-called "frustrated phagocytosis" (reversed endocytosis) is of particular importance as it gives a very high local extracellular concentration of granule constituents. It was first demonstrated on an IgG-coated surface (12). In this case the size of the "particle" can be regarded as infinite compared to the neutrophil and not phagocytizable. However, the granules move towards the part of the cell membrane which is attached to the opsonized surface and release their content into the micro-compartment between the IgG-coated surface and the cell membrane (12). It is easy to imagine that this will lead to tissue destruction *in vivo*, especially as the extracellular protease inhibitors may be excluded from the micro-compartment into which degranulation occurs. Such a mechanism of tissue injury is likely to be of importance in disease where immune complexes are deposited on structures like basement membranes.

In most of the above-mentioned examples

extracellular release of neutrophil enzymes is induced by phagocytosable or non-phagocytosable particles. Secretion of granule constituents can also be initiated by soluble compounds in the absence of particles. Thus C5a generated by activation of the complement system stimulates membrane fusion between granules and plasma membrane with subsequent extracellular secretion (10). It is not clear to what extent tissue damage can occur by this mechanism as the proteases released are rapidly diluted in the extracellular fluid and possibly inactivated by protease inhibitors.

Extracellular degranulation of neutrophils is governed by mechanisms involving cyclic nucleotides (44). Agents which raise the level of cyclic AMP inhibit release. Agents which lower the level of cyclic AMP or raise the concentration of cyclic GMP stimulate extracellular degranulation. The former effect is induced by adrenergic drugs, especially beta-adrenergic agonists, histamine and certain prostaglandins and the latter effect is induced by cholinergic drugs. However, the concentrations needed and the broad effects of the drugs limit their therapeutic role in modulation of neutrophil degranulation. Corticosteroids inhibit extracellular degranulation of neutrophils but high concentrations are needed. Therefore the anti-inflammatory effect of corticosteroids is difficult to explain on the basis of an inhibition of neutrophil release *in vivo*.

Below we will discuss the possible extracellular effects of neutral proteases of human neutrophils in the inflammatory process.

Effects on chemotaxis

Chemotaxis designates the directed movement of a cell as determined by a concentration gradient of chemoattractive substances (59). Chemokinesis describes alterations in the speed of cell movement both in directed and undirected migration. Most substances which attract neutrophils also increase the speed of cell movement but certain substances affect

only the speed without attracting the cells (59).

Some of the most potent chemoattractive substances are derived from the complement components C3 and C5 (55). It is therefore of interest that these components can be cleaved by human neutrophil proteases. The relative importance of this phenomenon is not established. Both the chymotrypsin-like enzyme (52) and granulocyte elastase (55) generate chemotactic activity from C3 and C5. In addition the chymotrypsin-like enzyme and elastase can inactivate preformed chemotactic activity produced both by C3 and C5 (55, 52). A C5-activating factor, distinct from chymotrypsin-like enzyme and elastase, has been found (60) in secondary neutrophil granules. It was proposed to be the predominant neutrophil-derived activator of C5 while azurophil granule proteases were suggested as predominant inactivators of C5 chemotactic activity.

Neutrophil proteases were found to be released also during chemotactic movement (50). Furthermore, chymotrypsin-like enzyme, depending on concentration, could both enhance and depress neutrophil migration. Similar effects were seen on both directed and undirected migration suggesting a chemokinetic effect of this enzyme. Chymotrypsin-like activity might, therefore, be involved in the chemotactic activation of neutrophils (50). Such a concept is supported by the finding that almost all chemoattractants described induce secretion of neutrophil constituents, which according to this concept should be a prerequisite for chemotactic activation. Thus it seems that neutrophil proteases can affect both the formation and degradation of chemoattractants and perhaps the activation of the neutrophil itself.

Effects on phagocytosis

The primary step in the phagocytic process is the recognition of the material which is to be phagocytized. The neutrophil is furnished with specific recognition units, which have their

counterparts in opsonically active plasma proteins. The two best-known opsonins are immunoglobulin and the C3b fragment of the third complement component (48). However, the neutrophil also recognizes foreign non-opsonized material via the so-called "non-specific" receptor (48). The "specificity" of C3b might be due to hydrophobic interaction with the neutrophil membrane. The contact between the Fc-part of the immunoglobulin and the "Fc-receptor" on the cell membrane has been suggested to be the main trigger for internalization. According to one hypothesis C3b is of importance for adherence of the particle to the cell surface which leads to accessibility of the "Fc-receptor" and subsequent internalization.

C3b can be formed by activation of the complement sequence but also as a direct effect of neutrophil proteases like elastase (17). In the latter case the neutrophil itself provides an amplification mechanism by which the cell bypasses the need of activation of the whole complement sequence to provide opsonically active C3 products.

Both chymotrypsin-like enzyme and elastase (13) do enhance the neutrophil ingestion rate of immune complexes because of a direct effect on the cell membrane. The results indicate that the chymotrypsin-like cationic protein changes the affinity of the "Fc-receptor" of the neutrophil membrane for immunoglobulin complexes. The net effect is therefore similar to that of opsonizing the immune complex with C3b. The chymotrypsin-like enzyme may change the properties of the "Fc-receptor" so that the need for C3b-opsonization is circumvented.

The effects of the neutral proteases on phagocytosis-related reactions summarized here indicate that the phagocytizing neutrophil by itself has the potential capacity to increase the effectiveness of particle engulfment.

Effects on coagulation and fibrinolysis

Fibrinogen is cleaved both by the

chymotrypsin-like enzyme (26), non-specific collagenase (29) and elastase (31). Specific degradation products are formed. Although distinct from those seen after plasmin digestion the products inhibit fibrinopolymerization similar to plasmin digested products (41). Several clotting factors are also digested by chymotrypsin-like enzyme and elastase (6). The finding of granulocytic proteases in plasma from patients with coagulation defects may therefore suggest a pathogenetic role for the proteases (6).

Attraction to and accumulation of neutrophils at the site of fibrin formation suggested a role of the neutrophil in fibrinolysis. This has been further substantiated by the demonstration of fibrin-fibrinogen degradation products as strong chemoattractants for neutrophils (47). Fibrin is readily dissolved by chymotrypsin-like enzyme, non-specific collagenase and elastase *in vitro* and the neutrophil activity in this respect may therefore be operative *in vivo* as an alternate pathway of fibrinolysis (41).

Effects on vasoactive systems

Both kinin-forming and kinin-destroying proteases (56) have been demonstrated in human neutrophils. The identity of these proteases is, however, not yet clear.

Subcutaneous injection in humans of minute amounts of chymotrypsin-like cationic protein produces an immediate vascular reaction (49). This reaction is probably not mediated by anaphylatoxin as granulocyte proteases do not form anaphylatoxin from human complement components (52). However, the chymotrypsin-like enzyme by itself seems to have an anaphylatoxin-like effect on human mast-cells (4), which might explain the vascular reaction of subcutaneous injection.

Effects on erythrocytes, lymphocytes and platelets

The cytotoxic action of the chymotrypsin-like enzyme is reflected by induction of increased membrane permeability of erythrocytes (15)

responsible for the haemolysis seen in washed blood which is stored.

Both the chymotrypsin-like enzyme and elastase stimulate B- and T-lymphocytes (11, 54). These effects are shared with other proteolytic enzymes and may involve a direct proteolytic attack on the cellular membrane. They could also be mediated indirectly via the effects of the proteases on other cell systems.

The chymotrypsin-like enzyme increases immune complex-mediated platelet aggregation and serotonin-release (14). This enzyme also induces "micro-aggregation" of platelets by an ADP-dependent mechanism and it competitively prevents thrombin-induced aggregation. A close relationship is therefore suggested between neutrophils and platelets in the inflammatory process.

CONCLUSIONS

The neutrophil provides major defence against microbicidal invasion. For this purpose it is equipped with a variety of antimicrobial and digestive agents. Most of these are potentially injurious not only to micro-organisms but also to the host. To avoid undesired consequences in connection with microbicidal destruction the host is provided with a number of protective mechanisms. The most important is to confine microbial destruction to intracellular phagosomes. Extracellular deposition of granule constituents can, however, occur by regurgitation during phagocytosis, reversed endocytosis and by secretion initiated by soluble compounds. The neutrophil proteases chymotrypsin-like cationic protein, elastase and collagenase may be most important for the extracellular action of human neutrophils. The chymotrypsin-like enzyme and elastase participate in the defence against gram-positive bacteria. These actions are exercised independent of the proteolytic activities as the latter can be abolished without interference with antimicrobial action. The cationic nature of these proteins is the basis for their antimicrobial action; another bactericidal and permeability increasing protein without pro-

tease activity has a great potency towards gram-negative bacteria.

Unrestricted release of neutrophil proteases due to reversed endocytosis may be the underlying mechanism of tissue destruction in certain immune complex diseases. Natural protease inhibitors are probably excluded from the micro-compartment between neutrophil and basement membrane, where degranulation occurs.

A generalized deficiency of protease inhibition as in α_1 -antitrypsin deficiency may increase the harmful effects of neutrophil proteases. Protective synthetic inhibitors active *in vivo* are still to be developed.

That extracellular degranulation can be harmful is without doubt. On the other hand, a limited secretion of neutrophil proteases might be of physiological importance for modulation of the inflammatory response. For instance, the neutral proteases can affect both the formation and degradation of complement-derived chemoattractants for neutrophils. These enzymes may actually be required for chemotactic activation of the neutrophil. The chymotrypsin-like cationic protein and elastase can also enhance the ingestion rate by increasing the affinity of the "Fc-receptor" of the neutrophil for immune complexes. In addition such proteases also provide a fibrinolytic system and stimulate B- and T-lymphocytes and platelet aggregation.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Cancer Society and the Swedish Medical Research Council.

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