Biol. Chem. Hoppe-Seyler, Vol. 377, pp. 217–226, April 1996 · Copyright © by Walter de Gruyter & Co · Berlin · New York

Review

# Pathogenic Mechanisms Induced by Microbial Proteases in Microbial Infections

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**Most bacterial and fungal proteases excreted into infected hosts exhibit a wide range of pathogenic potentials ranging from pain, edema or even shock to translocation of bacteria from the site of infection into systemic circulation, thus resulting in septicemia. The basic mechanism or principle common to all these phenomena is explained by kinin generation, either directly from high- and/or low-molecular weight kininogens or indirectly via activation of the bradykinin generating cascade: i.e. Hageman factor ➡ activated Hageman factor → prekallikrein ➡ kallikrein → high-molecular weight kininogen ➡ bradykinin. Some bacterial proteases are also involved in activation of other host protease zymogens such as plasminogen, procollagenase (matrix metallo proteases) and proenzymes of the clotting system. Furthermore, most bacterial proteases are not only resistant to plasma protease inhibitors of the hosts, most of which belong to a group of serine protease inhibitors called serpins (serine protease inhibitors), but they also quickly inactivate serpins. Some bacterial proteases may also activate bacterial toxins thus rendering toxigenic pathogenesis. They are also capable of degrading immunoglobulins and components of the complement system and facilitate propagation of micro organisms. All in all, microbial proteases are very critical in enhancing pathogenesis of severe diseases. It is also noteworthy that bacterial cell wall components themselves, i.e. endotoxin (or lipopolysaccharide) of gram negative bacteria and teichoic/lipoteichoic acid of gram positive bacteria, are also able to activate the bradykinin generating cascade – involving activation of Hageman factor as mentioned above.**

*Key words:* Bacterial translocation / Bacterial proteases / Bradykinin / Hageman factor / Protease inhibitors / Septicemia / Shock.

## Introduction

Many saprophytic bacteria produce large amounts of various proteases with broad substrate specificity such as *Pseudomonas* sp., *Serratia* sp., *Bacillus* sp., *Clostridia, Streptococci, Staphylococci*, etc. in order to meet nutritional demands, for instance for nitrogen. Another important nutritional component is carbohydrate, which is similarly generated by glycosidases such as amylase, hyaluronidase and others. In culture media poor in free nitrogen sources but rich in macromolecular proteins, their growth potential is very limited without protease production (Miyagawa *et al.*, 1991c). By adding either free amino acids or proteases to such culture media, the growth of protease deficient strains is enhanced (Miyagawa *et al.*, 1991b, c). On the other hand, many enteric bacteria are habitants in the environment (the intestine) which is rich in nutritional supply, and hence protease production could be minimal if any. However, without the production of proteases their pathogenic potentials were found to be much lower or even minimal as described below (Kamata *et al.*, 1985a, b; Miyagawa *et al.*, 1991a, b, c; 1994; Matsumoto *et al.*, 1996).

## Tissue Destruction

A most classic type of the disease mechanism caused by microbial proteases is the direct digestion and liquefaction of tissues of the infected foci. This was frequently observed in corneal keratitis caused by *Pseudomonas* sp. or *Serratia* sp. where the tissue lacks blood vessels which can supply defensive proteins and permit leukocyte migration.

We have demonstrated the cause-effect relation (or Koch's Postulates) of the corneal keratitis using serratial and pseudomonal proteases. Namely, the protease is the cause of pseudomonal and serratial keratitis: protease negative strains of *Pseudomonas* sp. did not cause the disease (Holder and Haidaris, 1979; Kamata *et al.*, 1985a, b; Miyagawa *et al.*, 1991a, b; 1994; Matsumoto *et al.*, 1996). In this connection we have observed that the broad specificity protease inhibitor, ovomacroglobulin (structurally homologous to human $\alpha_2$-macroglobulin, obtained from chicken egg white) inhibited keratitis caused by *Pseudomonas* sp. very effectively (Miyagawa *et al.*, 1991a, b; 1994). Furthermore, a combination of the antiprotease (ovomacroglobulin) with antibacterial agents in-

deed resulted in most beneficial outcome in therapy of *Pseudomonas*-infected cornea (Miyagawa *et al.*, 1994).

Periodontal disease is another hallmark event in direct tissue damage and activation of the bradykinin generating system (Scott *et al.*, 1993; Kaminishi *et al.*, 1993). A number of proteases are produced by *Porphyromonas gingivalis*. Among them a *P. gingivalis* collagenase (94 kDa) which undergoes fragmentation to yield multiple forms was described by Lowson and Meyer (1992). Other proteases [cysteine type, called gingipain, and metallo ($Zn^{++}$) type] have also been reported (Chen *et al.*, 1992; Hinode *et al.*, 1991; Scott *et al.*, 1993).

*Clostridia* sp. are known to produce collagenases which are so potent that they dissolve even blood vessels and bleeding would result quickly as they are injected subcutaneously, while hemolytic enzymes (lecithinases, lipases), which are also produced by these bacteria, induce red blood cells lysis. The hemoglobin release results in iron toxicity (Harber-Weis reaction) forming oxygen radicals which can be envisaged to occur at the site of microbial infection.

In addition to the direct action of microbial proteases, it is now evident that activation of other tissue damaging endogenous host protease zymogens by microbial proteases needs to be considered for damaging host tissues. It is known that fibroblasts, various types of cancer cells and macrophages can excrete proenzymes such as urokinase-type plasminogen activator (uPA) (Blasi, 1988; deBruin *et al.*, 1987; Hasui *et al.*, 1989; Marian *et al.*, 1990; Schlechte *et al.*, 1989; Conese and Blasi, 1995), or matrix metallo proteases (Blasi, 1988; deBruin *et al.*, 1987; Hasui *et al.*, 1989; Marian *et al.*, 1990; Matsumoto *et al.*, 1991) such as fibroblast procollagenase, type IV procollagenase, and stromelysins (Nagase *et al.*, 1990; Saari *et al.*, 1990). These various tissue-derived protease zymogens are activated by bacterial proteases (Uitto *et al.*, 1989; Sorsa *et al.*, 1992) as well as other stimuli such as superoxide (Weiss *et al.*, 1985; Saari *et al.*, 1990) and nitrogen oxides (reactive nitrogen intermediates) (Okamoto *et al.*, unpublished). Activation of plasminogen to plasmin, which also leads to activation of prekallikrein (and bradykinin formation) and of matrix metallo proteases (type IV collagenase and stromelysin) will cause degradation of tissue matrix and facilitate bacterial translocation. Plasminogen is also activated by plasma kallikrein (as described below) which has been activated by bacterial proteases (Akaike *et al.*, 1989). Activation of pro-uPA is facilitated by uPA receptor which exists on the cell surface of tumor cells, and uPA is neutralized by plasminogen activator inhibitor (PAI) (Conese and Blasi, 1995).

## Bradykinin Generating Cascade

Bradykinin (BK) is an endogenous peptide which exerts a wide range of pharmacological, physiological, and pathological effects in animals and man. BK is a nonapeptide released from high-molecular weight kininogen (HMW kininogen) due to proteolytic cleavage by plasma kallikrein as shown in Figure 1. Plasma kallikrein is generated from prekallikrein which usually undergoes activation by activated Hageman factor (activated factor XII, XIIa) (e.g. Figure 2). Another route which is probably less crucial is kallidin (decapeptide, Lys-BK) generation from low molecular weight kininogen (LMW kininogen) by tissue or glandular kallikreins. Kallidin may be converted to bradykinin by removal of the amino-terminal Lys by lysine aminopeptidase.

The most intriguing event in relation to bacterial and fungal infections is the activation of one or more steps of the bradykinin generating cascade: activation of either

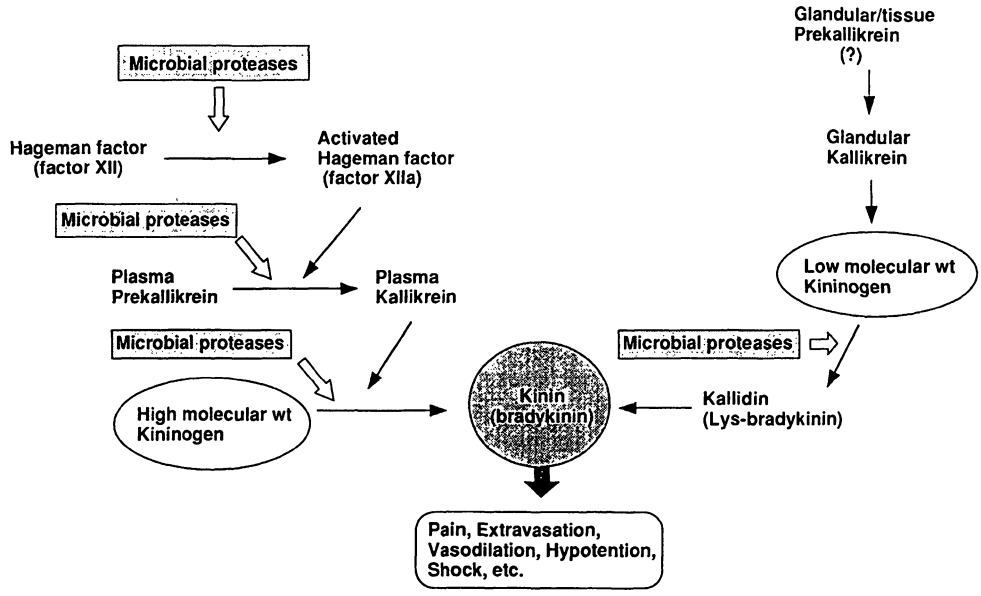


**Fig. 1** Role of Microbial Proteases in Activating Bradykinin Generating Cascade. Microbial proteases can activate at various steps.

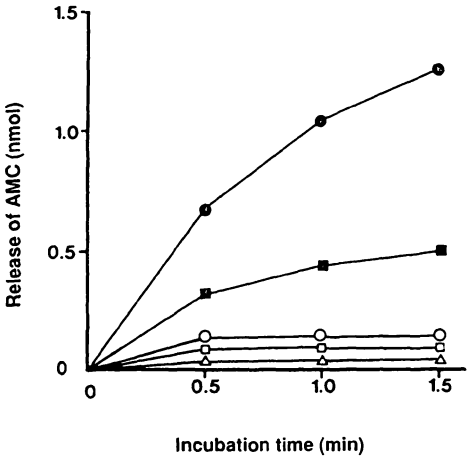


**Fig. 2** Activation of Plasma Prekallikrein by *Porphylomonas gingivalis* Protease.
The enzyme (●, 1.0; ■, 0.2 μg) was incubated with human plasma (20 μl) in the presence of Z-Phe-Arg-MCA, a substrate for plasma kallikrein. Open symbols indicate the incubation of plasma with 1 μg of the enzyme in the presence of (○) 1 μg of SBTI; (□) 2 μg of $\alpha_2$-antiplasmin. Control (△): 1 μg of pre-heated (100 °C, 5 min) protease (from Kaminishi *et al.*, 1993, with permission). SBTI does not inhibit *P. gingivalis* protease directly. Similar data were obtained with other proteases (Molla *et al.*, 1989a).

Hageman factor and/or prekallikrein and its direct action on LMW kininogen or HMW kininogen. This explains why pain and edema occur simultaneously at the site of infection.

The biochemical mechanism of this hallmark event of clinical manifestation remained rather unclear until about ten years ago. We have initially demonstrated using serratial 56 kDa protease as a model that the protease activates the bradykinin generating cascade in mammals (Figure 1 and 2) (Matsumoto *et al.*, 1984; Kamata *et al.*, 1985a, b; Maeda *et al.*, 1992; Molla *et al.*, 1989a). This finding was confirmed for many other bacterial and fungal proteases, some of which directly activate prekallikrein (Holder and Neely, 1989; 1992; Molla *et al.*, 1989a, b; Kamata *et al.*, 1985b; Kaminishi *et al.*, 1990; 1993; Nelly and Holder, 1990; Yamamoto *et al.*, 1990; Shibuya *et al.*, 1991; Tanaka *et al.*, 1992; Imamura *et al.*, 1994; 1995), which include various proteases from gram positive and negative bacteria such as *Serratia, Pseudomonas, Clostridium, Candida, Bacterioides, Porphylomonas, Staphylococci, Streptomyces, Bacillus, Vibrios, Streptococci*, etc. (Table 1). Some are metallo proteases, others are aspartate or cysteine proteases, and are produced by both anaerobes and aerobes. Universal for all these proteases is activation of one or more steps in the bradykinin generating cascade (Figure 1). Some of these proteases can generate bradykinin in human plasma even in the presence of serine protease inhibitors (Table 1). All of the eighteen microbial proteases which have been examined so far released bradykinin from purified HMW kininogen directly as well as from plasma (Molla *et al.*, 1987a; 1989a, b; Kaminishi *et al.*, 1990, 1993; Maruo *et al.*, 1993; Scott *et al.*, 1993; Khan *et al.*, 1993) (Table 1). Therefore, we may conclude that kinin generation is a universal event of bacterial infection (Maeda *et al.*, 1992). Activation of the bradykinin generating cascade is triggered not only by microbial proteases but also by a serine protease derived from house dust mite *(Dermatofagoides farinae)* (Maruo *et al.*, 1991; 1993). Furthermore, Holder and Neely (1989; 1990; 1992) showed that the bradykinin generating cascade is also associated with thermal burn and pseudomonal infection accompanying burn wounds. Another astonishing fact is that a few microbial proteases can directly generate bradykinin or kallidin from LMW kininogen as exemplified by *P. gingivalis, V. vulnificus* and *V. cholerae* in addition to the mite *(Dermatofagoides farinae)* serine protease.

**Table 1** Activation of Hageman Factor and Prekallikrein and Generation of Bradykinin by Various Microbial and Mite Proteases.

| Protease | Activation of | | Generation of kinin from | Generation of kinin from | Inhibited by | |
|---|---|---|---|---|---|---|
| | Hf | PK | HMWKNG | LMWKNG | CTI | SBTI |
| *Serratia* 56K protease | + | – | + | – | – | – |
| *Serratia* 60K protease | + | – | + | n.d.[a] | – | – |
| *Serratia* 73K protease | + | – | + | n.d. | – | – |
| *Pseudomonas aeruginosa* elastase | + | – | + | – | – | — |
| *Ps. aeruginosa* alkaline protease | + | – | + | – | – | – |
| *Aspergillus* protease | + | – | + | – | – | – |
| *Candida* protease | + | – | + | – | – | – |
| *Vibrio* protease | + | + | + | + | – | – |
| Subtilisin | + | + | + | + | – | – |
| Thermolysin | + | + | + | – | – | – |
| *Streptococcus pyogenes* protease | + | n.d. | + | n.d. | (n.d.) | (+)[b] |
| *Staphylococcus* V8 protease | – | – | + | – | + | + |
| *Streptomyces* protease | – | – | + | + | + | + |
| Df-protease[c] | + | + | + | + | + | + |

See ref. (Maeda and Molla, 1989 and others)
[a] not determined, [b] (+) weakly inhibited, [c] mite, *Dermatofagoides farinae*, serine protease.

## Systemic Effect of Kinin Generation

We (Khan *et al.*, 1993; 1994; Maeda *et al.*, 1996a, b; Shin *et al.*, 1996) showed the critical role of bradykinin generation in shock induced by pseudomonal and serratial proteases (Figure 3). Depletion of $\alpha_2$-macroglobulin from plasma enhanced sensitivity to septic shock induced by pseudomonal elastase (Khan *et al.*, 1994; 1995), and the plasma levels of Hageman factor and prekallikrein were lowered by the elastase. In this setting, infusion of $\alpha_2$-macroglobulin (Khan *et al.*, 1994; 1995), or soybean trypsin inhibitor (SBTI) (with longer plasma half-life due to polymer conjugation) (Shin *et al.*, 1996; Maeda *et al.*, 1996a, b) resulted in prevention of shock under conditions similar to septicemia.

Bacterial dissemination into the systemic circulation is also greatly enhanced by triggering kinin generation or suppressed by administration of appropriate protease inhibitors or kinin antagonists (Maeda *et al.*, 1993; Shin *et al.*, 1996; Maeda *et al.*, 1996a, b; Sakata *et al.*, 1996) (Figure 4). In addition to kinins, plasmin generation is considered to be a common mechanism of invasive dissemination by dissolving fibrin clots (Lottenberg *et al.*, 1994). This evidence supports a potential strategy for treatment of bacterial infections using appropriate protease inhibitors.

Flu symptoms caused by rhinovirus and influenza virus infections are now known to be a consequence of kinin generation (Proud *et al.*, 1990). More recently, various reports showed that periodontal diseases are heavily accompanied by kinin production and actions of proteases (Imamura *et al.*, 1994; 1995; Scott 1993; Travis *et al.*, 1995; Kaminishi *et al.*, 1993).

## Enhanced Kinin Generation in Cancer

Clinical manifestation of edema and pain as well as hypoalbuminemia and cachexia in cancer patients may be also associated with increased kinin generation most likely triggered by a plasminogen activator (Maeda *et al.*, 1988; Matsumura *et al.*, 1988; 1991). Using a plasma kallikrein inhibitor, SBTI, we tested whether it would inhibit

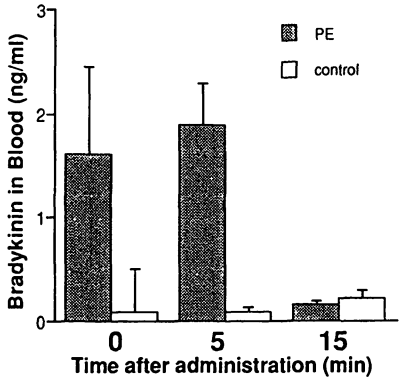


**Fig. 3** Generation of Bradykinin in the Circulation of Rats *In Vivo*.
*Pseudomonas* elastase (0.8 mg/kg) was injected intravenously.

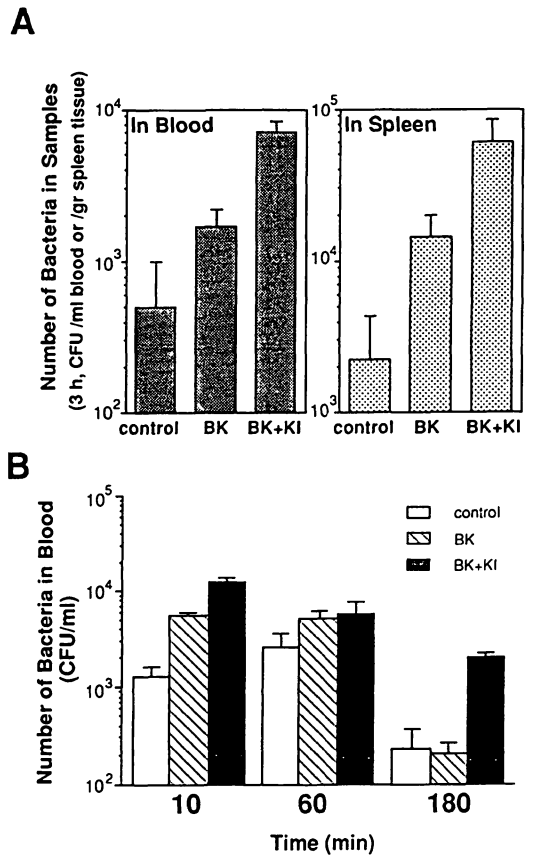


**Fig. 4** Bradykinin Facilitates Bacterial Translocation from the Peritoneal Cavity to the Circulation (Tail Vein) or to the Spleen in Mice.
Bacteria (*Vibrio vulnificus*, $3 \times 10^3$-colony forming units (CFU) per mouse, alone or with bradykinin [BK] (100 μg), BK (100 μg) + kininase inhibitors (KI) were injected intraperitoneally (*i.p.*). Kininase inhibitors used were enalapril and SQ20,881. Kinin receptor antagonist, HOE 140 (100 μg), was similarly injected *i.p.* and produced similar results. CFU (viable bacterial count) contained in 1 ml blood and in 1 g of spleen tissue was counted by colony formation on agar plate after cultivation.

fluid formation in mice bearing ascitic tumors. Indeed, SBTI inhibited fluid accumulation via suppression of bradykinin generation. However, augmentation of kinin activity by angiotensin I converting enzyme (ACE) inhibitors, which inhibit kininase II, resulted in a higher kinin concentration. These results are in accordance with recent findings and with the fact that $B_2$ (bradykinin) receptor antagonists (HOE140) also suppress ascitic fluid formation in mice (Maeda *et al.*, unpublished), which also suggest a possible beneficial use of kallikrein inhibitors and kinin antagonists for treatment of cancer patients, at least for the prevention of debilitation.

Formerly we found a new kinin in mammals, $^3$[hydroxyprolyl]-bradykinin ($^3$Hyp-BK), as the dominant form of kinin in ascitic or pleural fluid of cancer patients (Maeda *et al.*, 1988). In this kinin the proline residue at the 3rd position from the N-terminus is replaced by hydroxyproline. (It was later confirmed that $^3$Hyp-BK also occurs in HMW kininogen *in vivo*.) Quantifying the plasma levels of Hageman factor, prekallikrein and HMW kininogen, we found

that all these precursor levels were lower due to activation or consumption (Matsumura *et al.*, 1988; 1991). In support of this, bradykinin level in ascitic and pleural fluid caused by carcinomatoses was highly elevated (Matsumura *et al.*, 1991), indicating that fluid accumulation is due to bradykinin (and $^3$Hyp-BK). Although the dominant site of kinin generation is the tumor compartment, levels of bradykinin in plasma and ascitic/pleural fluid are similarly high due to rapid diffusion of small molecules into and out of the tumor compartment, and thus kinin effects are also exerted systemically. This may result in extravasation of fluid components and of proteins as well as in blood pressure decrease and hypoalbuminemia in cancer patients, and perhaps also in cachexia (Matsumura *et al.*, 1991; Maeda *et al.*, 1996b).

Immune response of cancer patients may result in production of various cytokines such as tumor necrosis factor (TNF), interferon-γ, interleukin-1, -2 (IL-1, -2) and other cytokines. Bradykinin is also known to stimulate release of TNF, IL-1 etc. from macrophages (Tiffany and Burch, 1989; Vandekerckhove *et al.*, 1991; Ferreira *et al.*, 1993). It is well known that bradykinin induces nitric oxide synthase (Palmer *et al.*, 1987), which results in the extravasation similar to bradykinin (Maeda *et al.*, 1994).

## Bacterial Cell Wall Components and Activation of the Bradykinin Generating Cascade

Negatively charged surfaces including glass, bacteria, acid clay, and liposulfatides can activate Hageman factor which leads to generation of bradykinin via the contact activation system (e.g. Shimada *et al.*, 1985). Gram negative bacteria contain lipopolysaccharide (LPS), the so called endotoxin, while gram positive bacteria contain lipoteichoic acid (or teichoic acid), poly-L-glutamate, poly-galacturonic acid etc. LPS and teichoic acid derivatives have been shown to be major stimuli for activation of Hageman factor and prekallikrein and thus also for kinin generation. Katori *et al.* (1989) found that the early phase of hypotension induced by LPS injection is blocked by SBTI and concluded that kallikrein plays a role. Similarly, Siebeck *et al.* (1993) showed the prevention of endotoxin shock by injection of aprotinin (Trasirol®). Obviously, LPS-induced hypotension is due to an increased expression of $B_1$ receptor caused by LPS treatment (Siebeck *et al.*, 1989). This hypotension could be prevented by kinin antagonists (Weipert *et al.*, 1988). Nagaoka and Katori (1975) reported earlier that extracorporal circulation device is responsible for kinin formation via activation of prekallikrein during open heart surgery, and kinin liberation was suppressed by aprotinin in this case. We have shown that gelatin-conjugated SBTI possessing a much longer *in vivo* elimination half life than native SBTI could suppress hypotension and shock more effectively than native SBTI (Shin *et al.*, 1996; Maeda *et al.*, 1996a, b). Therefore, it appears that bacterial cell wall components in addition to non-biocompatible materials can trigger the Hageman factor-kallikrein-kinin cascade. Hence, the counter measures to be envisaged include kallikrein inhibitors, kinin antagonists, and/or NO scavenger and nitric oxide synthase inhibitor (Figure 5).

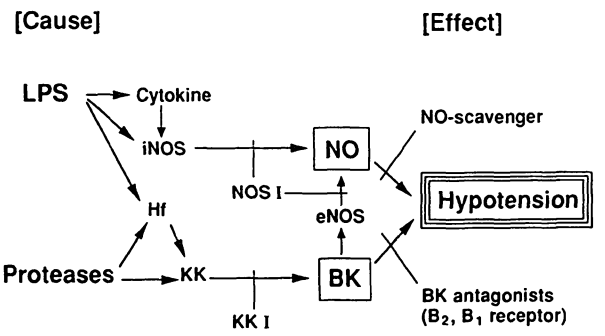


**Fig. 5** Relationship of Lipopolysaccharide (LPS) and Microbial Protease for Generation of Bradykinin and NO; an Universal Mechanism in Shock.
iNOS, inducible form of nitric oxide synthase; KKI, kallikrein inhibitor such as soybean trypsin inhibitor; eNOS, endothelial nitric oxide synthase; NOS-I, NOS inhibitor such as *N*-mono-methyl-L-arginine.

## Activation of Blood Coagulation, Fibrinolysis, Complement System and Neutrophils by Microbial Proteases

### Coagulation Cascade

Activation of Hageman factor and plasma kallikrein triggers liberation of other proteolytic enzymes *in vivo*, especially of the intrinsic coagulation cascade, leading finally to thrombin generation and thus to fibrin or clot formation. We have examined *Pseudomonas, Serratia*, and *Candida* proteases in this respect and found that activation of factor XII, XI, and finally of prothrombin to thrombin by these proteases occurs, which then converts fibrinogen to fibrin (e.g. Kaminishi *et al.*, 1994). Even more importantly, antithrombin III is readily inactivated by most of the microbial proteases (Table 2) thus leading to uncontrolled thrombus formation with severe consequences (hypercoagulation, disseminated intravascular coagulation syndrome or DIC).

### Fibrinolysis

Colman (1969) observed activation of plasminogen by human plasma kallikrein. Liberated plasmin then dissolves fibrin clots. Consequently, it is necessary to add *Serratia* 56K protease or another bacterial protease to the mixture of Hageman factor, prekallikrein, and plasminogen to achieve activation of plasminogen to plasmin (Akaike *et al.*, 1989), which is not only involved in fibrinolysis but also augments the infectivity of influenza virus 10–100 fold both *in vitro* and *in vivo*. Activation of plasminogen is important for bacterial spreading (Lottenberg *et al.*, 1994).

### Complement System

Activation of Hageman factor involves activation of the complement system (Ghebrehiwet *et al.*, 1983). Some

**Table 2** Inactivation of Human Plasma Protease-Inhibitors by *Serratia marcescens* 56K Protease and *Candida albicans* Protease (Maeda and Molla, 1989; Kaminishi *et al.*, 1995).

| Incubation time with proteinase (h) | % inactivation of inhibitors: (protease: inhibitor, ratio in mole) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Serratia marcescens* 56K | | | | | | *C. albicans* | |
| | $\alpha_1$-PI (1:200) | C1-inhibitor (1:50) | AT-III (1:25) | $\alpha_2$-AP (1:10) | $\alpha_2$-M (1:50) | ovo-M (1:50) | $\alpha_1$-PI (1:10) | $\alpha_2$-M (1:10) |
| 0.5 | 80 | 3 | 10 | 7 | 43 | 37 | 100 | 45 |
| 1 | 100 | 5 | 35 | 14 | 48 | 33 | n.d. | 65 |
| 2 | 100 | 15 | 90 | 31 | 62 | 28 | n.d. | 100 |
| 4 | n.d. | 64 | 100 | 68 | 70 | 23 | n.d. | 100 |
| 6 | n.d. | 100 | 100 | 90 | 80 | 21 | – | n.d. |
| 25 | n.d. | n.d. | n.d. | n.d. | 90 | 8 | – | n.d. |

bacterial proteases (e.g. *Serratia* 56K, and *Candida*) inactivate C1 inhibitor which might result in uncontrolled activation of the complement system (Molla *et al.*, 1989a, b; Oda *et al.*, 1990; Kaminishi *et al.*, 1995).

### Neutrophils

Plasma kallikrein and other bacterial proteases can stimulate neutrophils as described above. For instance, Wachtfogel *et al.* reported as early as 1983 that plasma kallikrein can release neutrophil elastase during blood clotting.

## Inactivation of Plasma Protease Inhibitors

The effect of microbial proteases on the various protease inhibitors in blood plasma is of special importance. Most of the above described pathological effects caused by microbial proteases including subsequent dysregulation of physiological functions by plasma proteases should not occur if the various protease inhibitors function normally. However, these inhibitors are easily degraded by the microbial proteases so that their inhibitory activity is lost rapidly (Morihara *et al.*, 1979; Travis and Salvesen, 1983; Potempa *et al.*, 1986; Maeda *et al.*, 1992). For instance, *Serratia* (56 K) protease can inactivate $\alpha_1$-protease inhibitor ($\alpha_1$PI), a major plasma serpin, at the enzyme (56K protease)/inhibitor ($\alpha_1$PI) ratio of 1/200, 80% in 30 min and 100% in 1 h (Table 2) (Molla *et al.*, 1986; 1989b). As $\alpha_1$PI is also inactivad by oxygen radicals derived from neutrophils at the site of inflammation (Travis and Salvesen, 1983) as well as by activated human neutrophil metallo protease (Desrochers *et al.*, 1992), elastase released from the neutrophils remains uninhibited and can thus provoke deleterious effects at the site of infection. Another example is inactivation of antithrombin (AT-)III (Table 2), which facilitates clotting and DIC together with excessive production of bradykinin leading to hypotensive state, a typical feature of septicemia. Table 2 summarizes these effects on various inhibitors. The broad spectrum serum inhibitor, $\alpha_2$-macroglobulin ($\alpha_2$M), is also degraded and inactivated by microbial proteases.

## Other Pathogenic Effects of Microbial Proteases

### Cytotoxicity of Bacterial Proteases

$\alpha_2$M forms E/I complexes involving a drastic conformational change and they are rapidly cleared from the circulation (Gonias and Pizzo, 1983). Many cells including fibroblasts, macrophages and various tumor cells have $\alpha_2$M receptors which recognize $\alpha_2$M in the E/I complex and the E/I complex will be internalized by an endocytotic process within 20–40 min. During prolonged incubation of such cells, the protease gradually inactivates $\alpha_2$M and is liberated from the complex within the cell. The free protease may disintegrate intracellular structures and thus lead eventually to cell lysis. This process was observed with *Serratia* 56K protease, *Pseudomonas* alkaline protease, and a few other microbial proteases (Maeda *et al.*, 1987a, b; 1989; Molla *et al.*, 1987a, b; Murakami *et al.*, 1992).

### Inactivation of Complement Factors and Chemotactic Activity

The complement system is one of the most potent defense systems and exhibits high bactericidal activity, which is coupled with recruitment of phagocytic cells (macrophages and neutrophils) by generating the chemotactic component, anaphylatoxin C5a. Hills *et al.* (1988) with a *Streptococci* B group-derived protease and we with a *Serratia* proteases (Molla *et al.*, 1989a, b) have demonstrated that streptococcal C5a protease as well as *Pseudomonas* and *Serratia* proteases inactivated complement function. Kaminishi *et al.* (1995) showed that *Candida* acid protease degrades especially C3 in human plasma. The chemotactic activity of C5a is also lost by treatment with *Serratia* protease (Oda *et al.*, 1990; Cleary *et al.*, 1992).

Host neutrophils and macrophages are attracted to migrate towards a certain kind of bacterial metabolic products, *e.g.* formyl-Met-Leu-Phe peptide. Since such formylated peptides are not produced by mammalian cells, the chemotactic response of the leukocytes to formylated peptides should be an excellent way to recognize and eliminate invading bacteria. Interestingly, pseudomonal

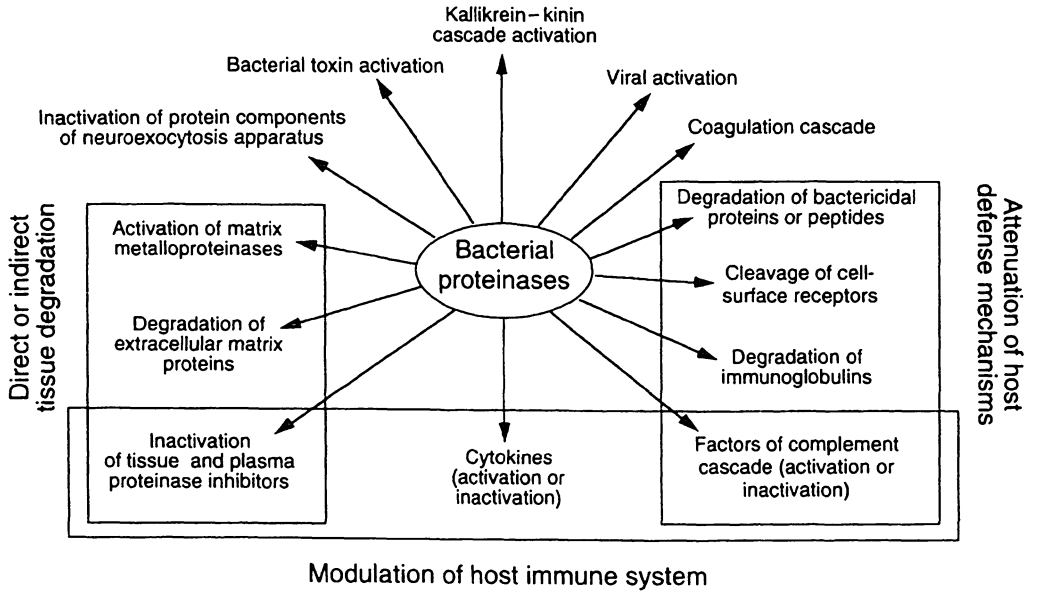


**Fig. 6** Multiple Functions of Microbial Proteases.
(From Travis *et al.*, 1995, with permission).

elastase interrupts this chemotactic response. The elastase not only hydrolyzes and inactivates formyl-Met-Leu-Phe peptide, but also destroys receptors on neutrophils for this peptide (Ijiri *et al.*, 1994). This seems to be the underlying cause of ring abscess formation in pseudomonal keratitis (Ijiri *et al.*, 1993; 1994).

### Activation of Bacterial Toxins

Several bacterial toxins are known to be activated by bacterial and host proteases, *e.g.* diphtheria toxin, anthrax toxin, pseudomonas exotoxin A, shiga toxin, shigalike toxins, botulinus toxin, cholera toxin, and tetanus and botulinus neurotoxins. A review article on these phenomena has appeared recently (Gordon and Leppla, 1994). The neurotoxins of both botulinus and tetanus were very recently found to be genuine proteases specific to acetylcholine receptors on neuronal cells (nerve ending; synaptobrevin) (Blasi *et al.*, 1993; Monecucco and Schiavo, 1993; Schiavo *et al.*, 1992).

### Inactivation of Immunoglobulin A and G

Many bacteria including *Streptococci, Neiseria, Hemophilus,* and *Bacterides* are able to produce so-called IgA proteases (Mulks, 1983; Plaut, 1983). We have used *Serratia* (56K) protease and found that it cleaves the hinge region of both IgA and IgG. When the susceptibility for proteolysis of different subclasses of these immunoglobulins were examined using *Serratia* (56K) protease, it caused efficient cleavage in the following order: $IgG_3 > IgA_1 > IgG_1 > IgG_2 > IgG_4 > IgA_2$ (Molla *et al.*, 1989b). Remarkably, this order rather coincides with the order of immunoglobulin-dependent complement activation for bactericidal activity. *Candida* protease also degrades Fc portion of IgG, thus abolishing opsonic activity, but Fab portion remains intact (Kaminishi *et al.*, 1995).

## Concluding Remarks

Microbial proteases not only exert pathological effects directly by destroying host tissues, they also potentiate inflammatory processes. Activation of the bradykinin generating system, inactivation of immunoglobulins, complement factors and complement-derived chemotactic factors are well known examples (Maeda and Molla, 1989). This leads to bacterial translocation and facilitates bacterial growth. Furthermore, microbial proteases activate endogenous host protease systems such as the clotting cascade and inactivate readily most of the plasma protease inhibitors. They are also able to activate many bacterial toxins and to lyse host cells via internalization of the enzyme-$\alpha_2$M complex. Figure 6 compiles our present knowledge on pathological events in which microbial proteases are involved. Some microbial and mite proteases also play a key role in augmenting influenza virus infectivity, thus in a complex infection by this virus and bacteria, much more complex etiological events may occur as discussed recently (Akaike *et al.*, 1989; 1994). Protease inhibitors and kinin antagonists thus seem to be potentially valuable therapeutic tools against the microbial infections.

## Acknowledgements

The authors would like to express sincere thanks to Professor Hans Fritz who gave invaluable suggestions in the preparation of the manuscript and Ms. R. Yoshimoto for typing. Research Grants to HM and TY from the Ministry of Education, Science and Culture of Japan are also gratefully acknowledged.

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