

行政院國家科學委員會專題研究計畫 成果報告

Cathepsin G 對多形核嗜中性白血球及單核球細胞免疫功能  
及生物活性的調控作用

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## Functional modulation of cathepsin G on polymorphonuclear neutrophils and mononuclear cells

### Abstract

Granular proteins of neutrophil have long been recognized as mediators of innate host defense. Newly discovered evidence suggests that granular constituents may also participate in adaptive immune response.

Cathepsin G (CG) is a highly cationic serine proteinase contained in the azurophilic granules of human polymorphonuclear neutrophils (PMN). A number of in vitro studies have revealed diverse putative functions of cathepsin G extracellularly including: (1) antimicrobial activity (2) regulation of inflammatory responses (3) degradation of extracellular matrix and (4) vasoregulation. However, its effect on immunological functions of PMN and mononuclear cells (MNC) have been rarely reported in the literature.

We use MTT test, RT-PCR, flow cytometry, ELISA and Western blot to investigate the effects of cathepsin G on immunological functions of PMN and MNC. We found that cathepsin G from 5 to 100 mU/ml did not affect the survival of PMN and MNC but effectively inhibit the phagocytosis of PMN. In addition, cathepsin G did not change the mRNA expression of inflammatory cytokines such as IL-1 $\beta$ , IL-4, IL-8, IL-10 and TNF- $\alpha$  in PMN. However, the expression of IL-2 and TNF- $\alpha$  mRNA was suppressed by CG. At the same time, cathepsin G enhanced the IL-8 production of PMN and MNC. The soluble IL-2 receptor of MNC was also increased by presence of CG. It is conceivable that IL-8 derived from macrophage facilitates PMN chemotaxis and activation. The increased release of soluble IL-2 receptor of T lymphocyte represents an activation marker of T lymphocyte. Moreover, cathepsin G could modulate cationic ion channel expression on the cell surface. CG increased the Na<sup>+</sup>-K<sup>+</sup>-ATPase expression on the surface of PMN and MNC but suppressed renal outer medulary K<sup>+</sup> channel (ROMK1) and modestly increased epithelial sodium channel (ENaC) expression in PMN. But the reverse finding in MNC was noted.

These results indicate CG exerts the immune regulation on PMN and MNC via the biologically functional modulation. In addition to the protease activity, CG plays a critical role in the adaptive immunity.

### Introduction

Cathepsin G, a neutral serine proteinase, is primary derived from azurophilic granules, but is also expressed in a proteolytically active membrane-bound form (1). Cathepsin G is also found in the granules of human monocytes and mast cells (2). It is referred to as a chymotrypsin-like enzyme because it hydrolyses peptide bonds after

leucine, methionine and phenylalanine. However, cathepsin G is considered to be a rather inefficient proteinase, degrading collagen and proteoglycan more slowly than neutrophil elastase (3). Various physiological effects are ascribed to cathepsin G: antimicrobial activity, degradation of extracellular matrix, vasoregulation (3), activation of neutrophil elastase (4) and processing of IL-8 (5). In addition, cathepsin G mediated the monocyte chemotactic activity (6) via the IL-8-induced degranulation and release of neutrophil azurophilic and specific granule makers (7). Cathepsin G is a more potent chemoattractant for monocytes than azurocidin or thrombin (7). Moreover, cathepsin G was also found to be an equally potent chemoattractant for neutrophils, but not for lymphocytes. Under modified by diisopropyl fluorophosphates or phenylmethanesulfonyl fluoride (PMSF), the inhibition of proteolytic activity of cathepsin G inactivated its monocyte chemoattractant activity, suggesting that the proteolytic activity of cathepsin G is essential for its chemotactic activity. Earlier studies of leukocyte chemotaxis revealed the involvement of chymotrypsin-like protease in regulation of leukocyte motility (8,9). Lomas et al. found that butyryl-L-phenylalanine-L-antichymotrypsin and antibodies to cathepsin G inhibit fMLP and C5a-induced chemotaxis (10). These results suggested a role of cathepsin G in regulation of leukocyte chemotaxis but the physiological relevance of the involvement of this protease(s) in chemotaxis remains unclear.

## Results

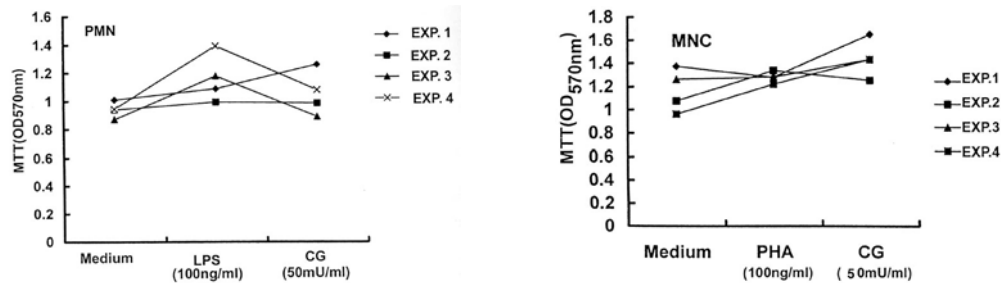


Fig. 1 Cathepsin G exert no significant effect on survival of PMN and MNC by MTT assay

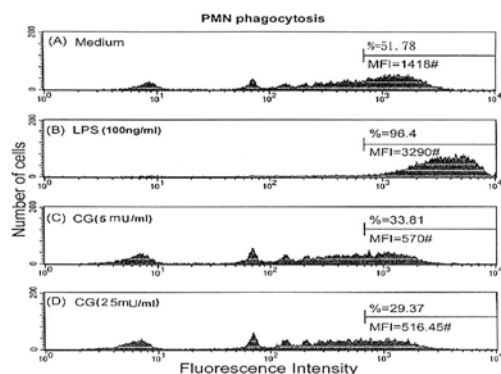


Fig.2 Cathepsin G significantly suppresses PMN phagocytosis

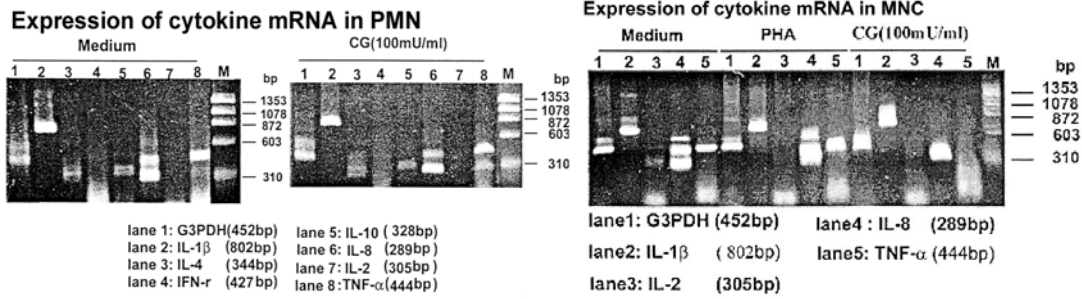


Fig. 3 Cathepsin G exert no significant effect on the expression of cytokine mRNA in PMN but suppressed the expression of IL-2 and TNF- $\alpha$  mRNA in MNC

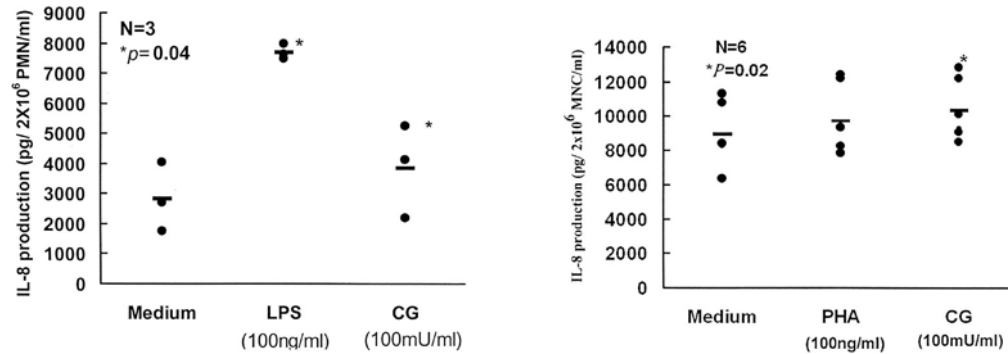


Fig. 4 Cathepsin G enhanced the IL-8 production of PMN and MNC

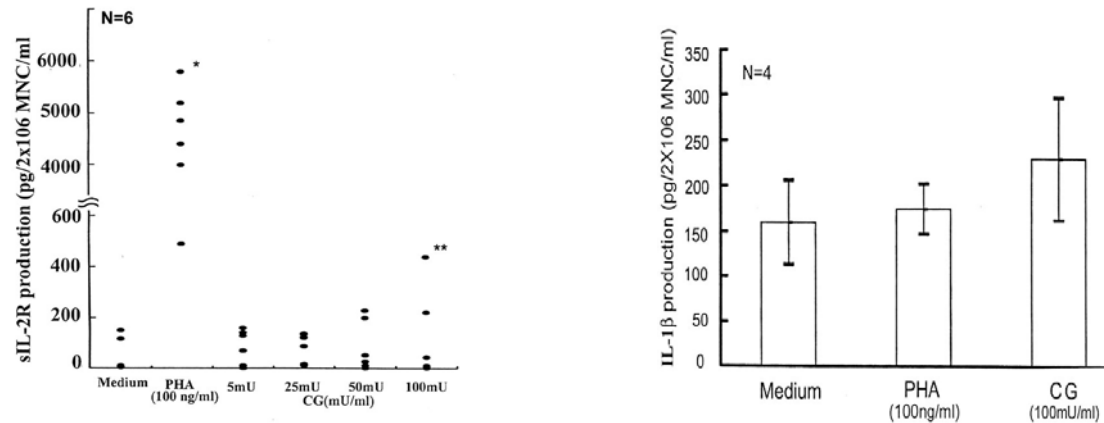


Fig.5 Cathepsin G enhanced the aIL-2R and IL-1 $\beta$  production of MNC

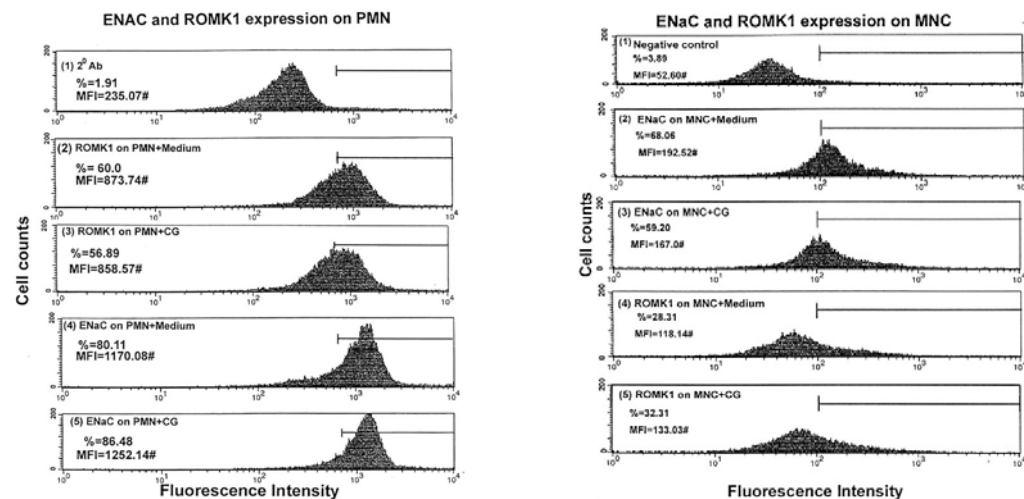


Fig. 6 Cathepsin G exert no significant effect on membrane expression of ENAC and ROMK1 on PMN and MNC

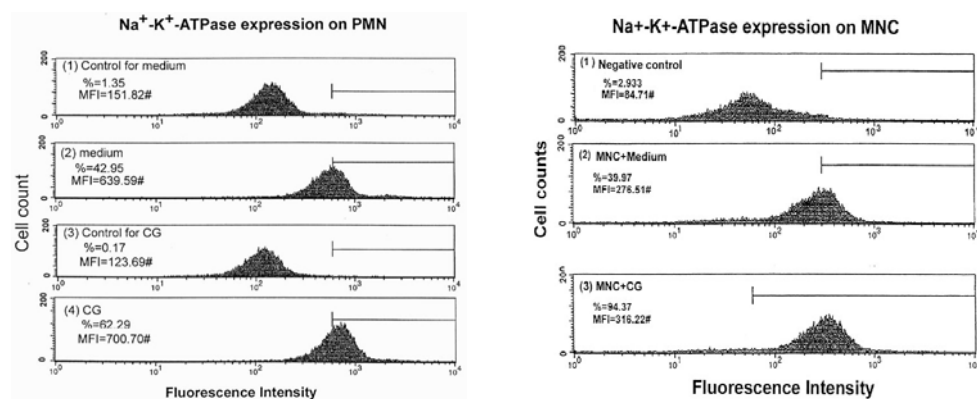


Fig.7 Cathepsin G enhanced the membrane expression of Na<sup>+</sup>-K<sup>+</sup> ATPase on PMN but no MNC

### Discussion

The group of T. Ley (11) reported that neutrophils from cathepsin G<sup>-/-</sup> mice have normal morphology and azurophil granule formation, and display normal phagocytosis and superoxide production but do have a decrease in wound-breaking strength (12). The cathepsin G is not essential for mammalian development but it plays a role in neutrophil trafficking. In addition, cathepsin G is a potent chemoattractant for monocytes and polymorphonuclear neutrophils (PMN); the immune defects may be, at least partially, attributed to insufficient release of cathepsin G in neutrophil, which is necessary for proper development of an inflammatory response. Interleukin 8, the central molecules of neutrophil activation via the autocrine and paracrine mechanism, is the potent chemotactic and activating factor for PMN. Our finding suggests that cathepsin G modulates the immune response of PMN in inflammation via the enhanced IL-8 production of PMN and MNC.

Chetov O (6) reported cathepsin G is a potent chemokinetic stimulant for T lymphocytes. Based on these data and the fact that human cathepsin G injection into murine skin induced infiltration of neutrophils and mononuclear cells, cathepsin G may serve as a neutrophil-derived signal that induces subsequent mononuclear cell and T-cell-dependent immune responses (1). Additionally, cathepsin G is mitogenic for murine T cells (1), in agreement with previous reports that human cathepsin G can increase DNA synthesis in both human T and B cells and bind to human T cells, B cells and natural Killer cells (13,14). The findings of inactivation of cathepsin G enzymatic activity by PMSF completely abolishing its proliferative activity for murine spleen cells (1) agrees with the reports of proteolytically active cathepsin G

necessary for its stimulation of murine B (15) cells and human lymphocytes (13,14). Moreover, co-administration of cathepsin G with an antigen (KLH) to mice shows cathepsin G to have immunoadjuvant activity (16). Thus, cathepsin G enhances both Th1 and Th2 limbs of the immune response. Our studies also revealed the potent enhancer of cathepsin G in immune response of mononuclear cells (MNC) via cytokine production, especially IL-8. Thus, IL-8 could be the key cytokine in the cathepsin G mediated immune response between PMN and MNC. Moreover, these immune responses may be mediated by the activation of membrane molecules of Na<sup>+</sup>-K<sup>+</sup> ATPase increased by the presence of cathepsin G. Therefore, the active modulator of immune response of cathepsin G also clarifies the presence of this weak and slow-acting granular protease in PMN.

In addition to the professional phagocytes, PMN also play a central role in the network of immune system via the response and/or production of cytokines. Granular proteinases of PMN mediate not only the traditional intracellular/extracellular destruction, but also involve the process of chemotaxis, cytokine processing and stimulation of lymphocytes. Our result further elucidate that the cathepsin G, one of the granular proteinases, engage in the adaptive immunity via the modulating the biological/ immunological functions of PMN and MNC. Interleukine 8 might be the critical cytokine mediating the functional modulation.

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