Protease-induced leukocyte chemotaxis and activation : Roles in host defense and inflammation

Kenji Tani, Fumitaka Ogushi, Teruki Shimizu, and Saburo Sone

Third Department of Internal Medicine, The University of Tokushima School of Medicine, Tokushima, Japan

Abstract: The migration of leukocytes such as neutrophils, monocytes and lymphocytes into inflamed lesions is one of the critical events of inflammation. Although the traditional function of neutrophil-derived antimicrobial proteases is to ingest and kill bacteria, some neutrophil serine proteases have been shown to induce leukocyte migration and activation. Mast cell-derived chymase also has the chemotactic activity for leukocytes. During the acute phase of inflammatory and allergic diseases, the predominantly migrated cells are neutrophils and mast cells, respectively, and in the subsequent chronic phase, monocytes and lymphocytes are mainly migrated. The chemotactic activity for monocytes and lymphocytes of neutrophil-derived serine proteases and mast cell-derived chymase may have a role in switching acute inflammation to chronic inflammation and delayed-type hypersensitivity. Recently, aminopeptidase N and endothelin were shown to induce chemotactic migration of leukocyes. Thus, protease-induced leukocyte chemotaxis and activation may play an important role in immunologic events of inflammatory and allergic diseases. J. Med. Invest. 48 : 133-141, 2001

Keywords **:** *chemotaxis ; leukocyte ; thrombin ; cathepsin G ; chymase ; endothelin ; aminopeptidase N*

INTRODUCTION

Leukocyte infiltration from a blood compartment into inflammatory sites is one of the characteristic elements of the inflammatory process. Locally produced chemotactic agents were suggested to play a crucial role in the leukocyte accumulation. Several members of a newly described family of chemotactic cytokines called chemokine are now believed to have an important role in inducing the migration of leukocytes (1-3). On the other hand, some proteases have been recently reported to induce chemotactic migration of leukocytes (summarized in Table 1). Originally, thrombin, a trypsin-like serine protease involved in blood clotting generated during vascular injury, was shown to have chemotactic activity for monocytes and neutrophils (4, 5). Recent studies have reported that various serine proteases can induce leukocyte chemotaxis. For example, CAP37/azurocidin was reported to have chemotactic activity for monocytes (6) and lymphocytes (7). We showed that cathepsin G, chymase and endothelin induce chemotactic migration of neutrophils and monocytes (8-10). In addition to serine proteases, we recently demonstrated that aminopeptidase N can induce chemotactic migration of T lymphocytes (11). This article describes recent studies on protease-induced leukocyte chemotaxis and activation, and their role in inflammatory and immunological disorders.

Received for publication July 5, 2001 ; accepted August 2, 2001.

Address correspondence and reprint requests to Kenji Tani, M.D., Third Department of Internal Medicine, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-633-2134.

NEUTROPHIL CATHEPSIN G

Human cathepsin G is an antimicrobial chymotrypsin-like enzyme that is found in the azurophil granules of human neutrophils, monocytes and spleen cells

Table 1. Proteases with chemotactic activity for leukocytes

Preteases	Cell source (s)	Target cells
Thrombin		Monocytes
		Neutrophils
CAP37/azurocidin	Neutrophils	Monocytes
		Lymphocytes
Cathepsin G	Neutrophils	Monocytes
	Monocytes	Neutrophils
	Mast cells	
Endothelin	Neutrophils	Monocytes
	Endothelial cells	Neutrophils
Chymase	Mast cells	Monocytes
		Neutrophils
Aminopeptidase N	Monocytes	Lymphocytes (CD4+>CD8+)
	Macrophages	
	Fibroblasts	

(12) and comprises 18% of the azurophil granule protein (13). Cathepsin G exerts a broad-spectrum of antibacterial action *in vitro* against Gram-negative and positive bacteria independent of its serine protease activity. In addition to the antimicrobial activity, cathepsin G can induce degradation of extracellular matrix, vasoregulation, activation of neutrophil elastase, and cytokine processing (13).

Chemotactic activity

Recently, Chertov *et al*. showed that cathepsin G has the chemotactic activity for monocytes and neutrophils (8). The chemotactic effect appears in a dose-dependent manner with an optimal concentration of 0.5-1 μg/ml. The monocyte chemotactic activity of cathepsin G is more potent than thrombin. Subcutaneous sites of cathepsin G injection in mice were infiltrated by these inflammatory cell types (8). The enzymatic activity of cathepsin G is required for its *in vitro* and *in vivo* chemotactic activity because inactivation of cathepsin G-enzymatic activity by inhibitors for serine proteases abolished the chemotactic activity.

T cell activation

We recently found that cathepsin G significantly stimulated antigen-specific Ig antibody production in association with activation of, and cytokine production by, murine T cells (14). The injection of cathepsin G into mice increased serum antigen-specific IgG1 and IgG2a subclasses *in vivo* (Fig. 1). *In vitro* restimulation of lymph node cells from immunized mice with antigen showed that cathepsin G increased antigen-specific lymphoproliferative responses and induced a marked increase in interferon (IFN)-γ production (Table 2). IFN-γ is responsible for the cathepsin G-enhanced antigen-specific IgG2a response of the immunized mice because IFN-γ, a Th-1 cytokine, is known to be associated with cell-mediated immunity and the preferential induction of IgG2a (15, 16). Cathepsin G also increased antigen-specific production of the Th2 cytokine, interleukin (IL)-4 (Table 2). This may account for the antigen-specific IgG1 production because IL-4 is known to be critical for the expansion of Th2 responses characterized by increased synthesis of IgG1 (17). These results suggest that neutrophil cathepsin G may have a role in the regulation of lymphocyte-dependent immunological reactions. Previous studies reported that human cathepsin G selectively stimulates both human T and B lymphocytes (18) and increases IFN-γ production by T cells (14). The mitogenic activity of cathepsin G was dependent on its enzymatic activity since pretreatment with diisopropylfluorophosphate (DFP) ablated the enhancement of proliferation. These results suggest that enzymatically active cathepsin G up-regulates antigen-specific Ig antibody production, and may act as an immune adjuvant in addition to possessing antibacterial action. Although Yamazaki and Aoki showed that cathepsin G exhibits specific binding to human lymphocytes (19), the receptor for cathepsin G has not been determined.

CAP37/AZUROCIDIN

Cathionic antimicrobial protein (CAP) of MW 37 kD, also termed azurocidin, was first isolated and purified from the granules of human neutrophils by Shafer *et al*. in 1984 (20). CAP37/azurocidin is a serine

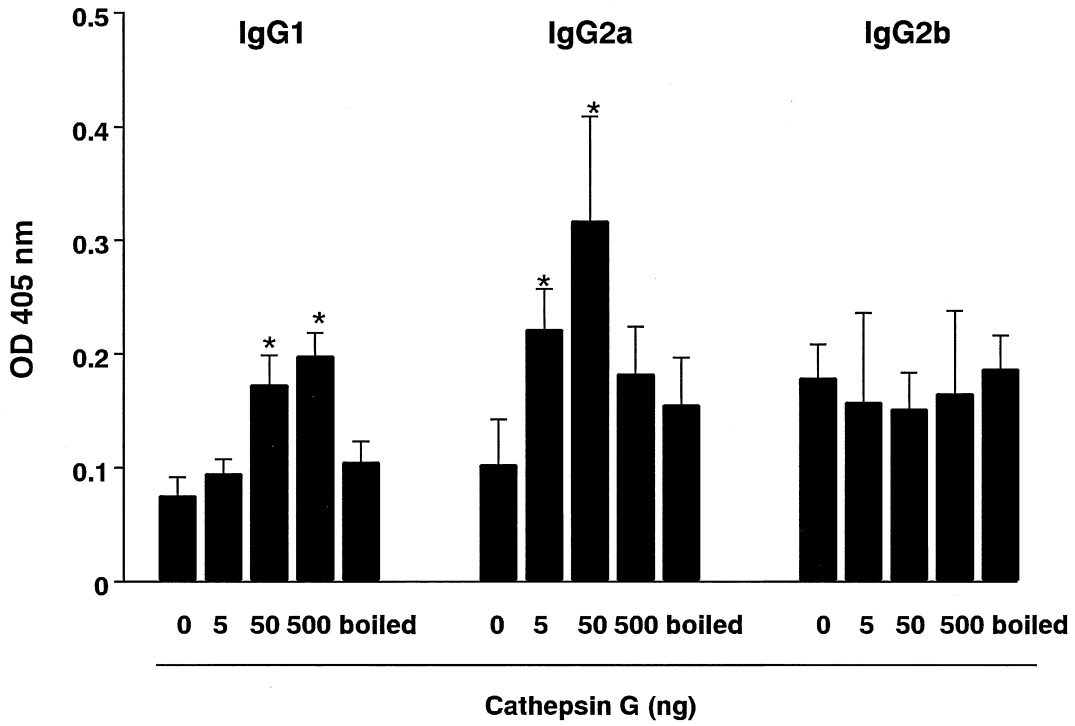

Fig. 1 IgG1 and IgG2 levels of serum keyhole limpet hemocyanin (KLH)-specific antibodies were increased by administration of human cathepsin G. Mice were immunized s.c. with 50 μg of KLH on Day 1, and treated s.c. with 5-500 ng cathepsin G, 500 ng boiled cathepsin G, or PBS alone daily from Day 1 to 5. On Day 10 KLH-specific antibodies in sera were assayed by ELISA. Values show means and SD of measurements from three mice. *indicates statistically significant differences ($p<0.05$) relative to mice treated with KLH alone.

Table 2. Effect of cathepsin G on KLH-induced cytokine release by lymph node cells from immunized mice[a]

in vivo treatment	IFN-γ (pg/ml)	IL-4 (pg/ml)
KLH + PBS	47.0 (21.6)	231.3 (33.1)
KLH + cathepsin G (5 ng)	159.3 (38.7)[b]	500.7 (61.5)[c]
KLH + cathepsin G (50 ng)	224.3 (22.4)[c]	860.0 (146.1)[c]
KLH + cathepsin G (500 ng)	289.3 (42.7)[c]	651.0 (126.6)[c]
KLH + boiled cathepsin G	32.7 (9.8)	285.7 (26.1)

[a] Mice were immunized s.c. with 50 μg KLH along with various doses of cathepsin G, 500 ng of boiled cathepsin G or PBS alone. After 10 days, single cells prepared from draining lymph nodes were incubated with 50 μg/ml of KLH *in vitro*. After 48h, culture supernatants were harvested, and assayed for IFN-γ and IL-4 levels by ELISA. Values means (SD) from three mice.
[b] Significantly different from the values of mice treated with KLH alone ($p<0.05$)
[c] Significantly different from the values of mice treated with KLH alone ($p<0.01$)

protease homolog exhibiting 45% sequence indentity to neutrophil elastase, and 30-37% identity to several other granule serine proteases (21).

Chemotactic activity

CAP37/azurocidin was shown to be a potent chemoattractant for monocytes in 1990 (6). This monocyte chemotactic activity is 80-100% that of N-formyl-methionyl-leucyl-phenylalanine (fMLP). Later, Flodgaard *et al.* demonsterated that CAP39/azurocidin has chemotactic activity for fibroblasts (22). The bactericidal activity of CAP37/azurocidin occurs at acidic pH whereas its chemotactic activity occurs at neutral pH. Moreover, the concentration of CAP37/azurocidin required for chemotaxis is significantly less than that required for antibacterial activity. Recently, Chertov *et al.* showed that CAP37/azurocidin induced chemotactic migration of T lymphocytes *in vitro* and *in vivo* (7). CAP37/azurocidin represents the T cell chemotactic activity at nanomolar concentrations though it carries out the antimicrobial activity at micromolar concentrations.

CHYMASE

Chymase is a major chymotrypsin-like serine protease expressed in the secretory granules of mast cells in many mammalian species (23). It was recently clarified that chymase was involved in the inflammatory processes of a variety of diseases. Chymase degrades constituents of extracellular matrix by the activation of matrix metalloprotease (MMP)-1 and MMP-3 (24), suggesting that this enzyme participates in the pathogenesis of matrix degradation in the rheumatoid joint, at sites of tumor invasion, and in human atherosclerotic lesions. Mizutani *et al.* showed that human chymase can convert an inactive precursor 31 kD IL-1 β to a biologically active IL-1 β (25). On the other hand, chymase stimulates secretion from cultured airway submucosal gland serous cells (26).

Chemotactic activity

Human chymase was recently shown to have the potent chemotactic activity for monocytes and neutrophils in concentration ranging from 0.1 to 10 μg/ml as well as human cathepsin G (Fig. 2A and 2 B). The activity was as potent as that of fMLP. The proteolytic activity of chymase participates in the chemotactic activity. Although mast cells have been reported to produce chemokines, such as macrophage inflammatory protein (MIP)-1α, MIP-1β and monocyte chmoattractant protein (MCP)-1 (27), which may induce leukocyte migration into the inflammatory lesions, mast cell chymase may be one of the candidates for mast cell-derived chemoattractants for inflammatory cells. A recent report demonstrated that the injection of chymase into mice induced the accumulation of neutrophils and eosinophils at the injection site (28). Findings about chymase-induced chemotaxis indicate that chymase can directly induce chemotaxis of leukocytes into the region.

ENDOTHELIN

Endothelin (ET) was first isolated from culture medium of porcine endothelial cells and shown to be a vasoconstrictor (29). ET-1 (1-31) is a novel 31-amino acid length peptide derived from big ET-1 by chymase or other chymotrypsin-type proteases, and is a predominant ET peptide in human neutrophils (30). Cathepsin G can transiently convert big ET-1 to ET-1 (1-31) and can degrade ET-1 (1-31).

Chemotactic activity

Recently, ET-1 (1-31) was reported to exhibit chemotactic activity toward neutrophils and monocytes (10). The functions of ET are known to be mediated by two distinct subtypes of receptors, ETA and ETB receptors. The chemotactic effect of ET-1 (1-31) may be mediated by ETA receptor because the chemotactic effects and an increase in intracellular free Ca2+ caused by ET-1 (1-31) were inhibited by the ETA receptor antagonist but not by the ETB receptor antagonist. ET-1 (1-21) was also reported to have the chemotactic effect (31) but the chemotactic effect of ET-1 (1-31) was considerably greater than that of ET-1 (1-21).

AMINOPEPTIDASE N

Aminopeptidase N is a membrane-bound metalloprotease, and was shown to be identical to CD13 (32), a 150-kD cell surface glycoprotein, which was originally used as a marker for subpopulations of hematopoietic cells (33). CD13/aminopeptidase N is widely distributed in a variety of mammalian cells such as monocytes/macrophages, fibroblasts, neutrophils, endothelial cells and epithelial cells (34, 35). This peptidase was shown to be involved in the degradation of extracellular matrix in tumor invasions (36) and the processing of peptide for presentation by antigen-presenting cells (37). Although little information is available concerning the regulation of CD13/aminopeptidase N expression in human diseases, recent studies have reported that lymphokines such as IFN-γ and IL-4 up-regulate the expression of CD13/aminopeptidase N in all cell types (34, 38).

Chemotactic activity for T lymphocytes

We recently reported that CD13/aminopeptidase N induces chemotactic migration of human lymphocytes (11) (Fig. 3). Chemotactic activity induced by CD13/aminopeptidase N is equivalent to that of 50 ng/ml of MIP-β. The enzymatic activity of CD13/aminopeptidase N is necessary in its chemotactic activity because treatment with bestatin, a specific inhibitor for aminopeptidases, reduces the chemotactic activity for lymphocytes. CD13/aminopeptidase N also manifested chemotactic activity for purified human CD4+ and CD8+ T lymphocytes, but the response of CD4+ T cells was greater than that of CD8+ T cells. Since CD13/aminopeptidase N does not induce chemotaxis of monocytes or neutrophils, CD13/aminopeptidase N shows chemotactic activity spe-

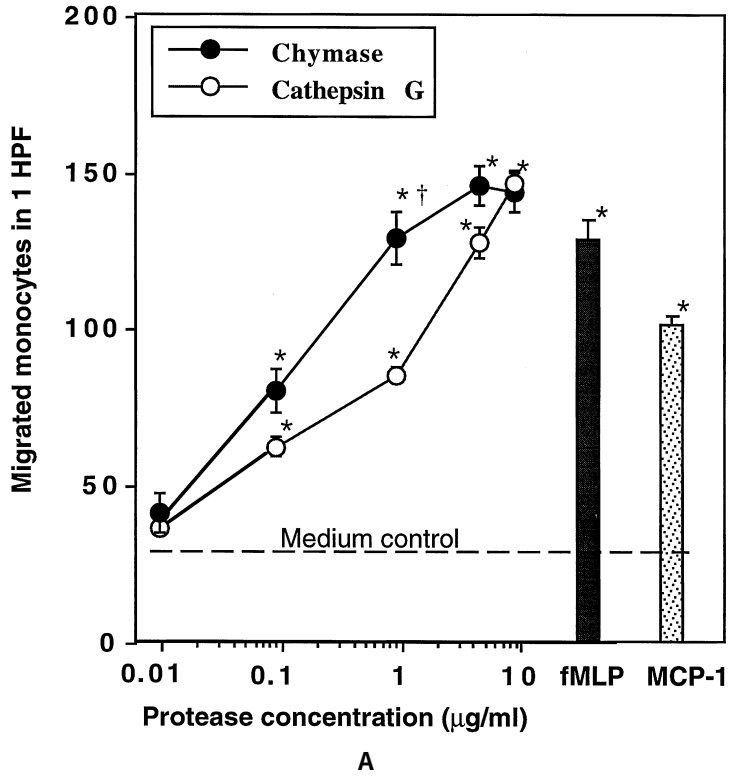

Fig. 2. A) Monocyte migration induced by chymase and cathepsin G. Monocytes were separated from the peripheral blood of healthy donors by centrifugal elutriation in a Beckman JE-5.0 elutriation system. The results are expressed as the number of migrated cells in 1 HPF. Fifty ng/ml of MCP-1 and 10^{-8} M of fMLP were used as positive controls for monocyte chemotaxis. * and † indicate significance of differences for comparing the value of medium control ($p<0.05$) and for comparing the value of cathepsin G ($p<0.01$), respectively.

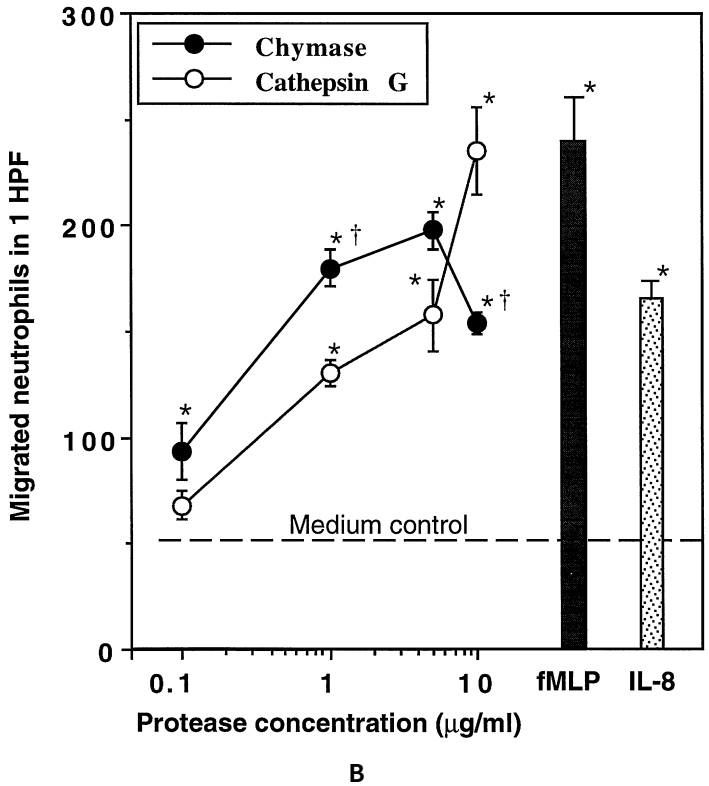

Fig. 2. B) Neutrophil migration induced by chymase and cathepsin G. Neutrophils were purified from the peripheral blood of healthy donors by the sedimentation method. The results are expressed as the number of migrated cells in 1 HPF. Fifty ng/ml of IL-8 and 10^{-8} M of fMLP were used as positive controls for neutrophil chemotaxis. * and † indicate significance of differences for comparing the value of medium control ($p<0.05$) and for comparing the value of cathepsin G ($p<0.05$), respectively.

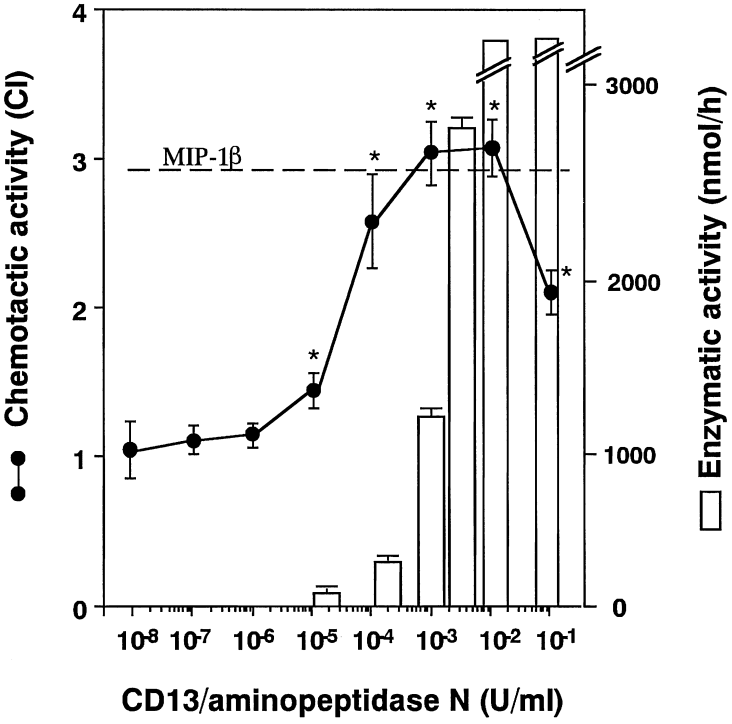

Fig. 3. Chemotactic and enzymatic activities of CD13/aminopeptidase N for human lymphocytes. Lymphocytes were separated from the peripheral blood of healthy donors by centrifugal elutriation in a Beckman JE-5.0 elutriation system. MIP-1β is included as a positive control. The results are expressed as the chemotaxis index (CI) which represents the ratio of the number of cells in HPF in the test to control samples (medium control). Columns show the enzymatic activity of CD13/aminopeptidase N which was assayed fluorometrically with L-leucine-AMC as a substrate and was expressed in nanomoles of substrate cleaved per hour. Results are expressed as means±SEM. * indicates significantly different from the value of medium control ($p<0.05$).

cific for lymphocytes.

CLINICAL SIGNIFICANCE

Neutrophil-derived serine proteases

Infiltrations of monocytes and lymphocytes into inflamed lesions are normally preceded by an initial influx of neutrophils. Neutrophil-cathepsin G may have a role in the development of further accumulation of neutrophils and monocytes into inflammatory sites by its chemotactic activity. Antibiotic proteases such as cathepsin G and CAP37/azurocidin secreted by neutrophils play a major role in resistance to the early stages of infections by killing microbes (13, 39). Following phagocytosis of microbial agents or other particulate substances, these proteases are released from the granules into phagocytic vacuoles and also into the extracellular milieu (40). Kudo *et al.* showed that depleting rat neutrophils by treatment with anti-rat neutrophil antiserum reduced subsequent development of chronic delayed-type hypersensitivity reactions (41, 42). Similarly, it has also been reported that suppression of neutrophil migration to inflammatory sites by infusion of anti-IL-8 mAb decreased not only acute inflammatory responses (43) but also delayed-type hypersensitivity responses (44). Recently, it was demonstrated that IL-8, a chemokine with potent chemotactic and activating effects on human neutrophils, induced neutrophil accumulation at the injection site followed by T cell infiltration in SCID mice administered human T cells (45). These results suggest that neutrophils could release chemoattractants that mediate T cell accumulation at sites of inflammation. We also showed that cathepsin G up-regulates antigen-specific Ig production as a result of T cell activation (14). Taken together, neutrophil cathepsin G and CAP37/azurocidin may play a role in the communication between cell types involved in innate or natural resistance mediated, for example, by neutrophils and those responsible for adaptive immunity such as T and B cells.

Mast cell chymase

When mast cells are stimulated by inflammatory mediators, mast cells increase in number in the lesion and release a variety of chemical mediators and proteases into the extracellular environment (46). The role of mast cells in immediate-type reactions of allergic diseases has been extensively studied, but the role in delayed-type reactions has not been understood. Although the late phase reactions in allergic disorders such as bronchial asthma are associated with enhanced leukocyte infiltration at sites of allergen challenge, the striking recruitment of leukocytes are incompletely understood in mechanical terms. Findings about chymase-induced leukocyte chemotaxis suggest that chymase released from mast cells in immediate-type reactions may play a role in the ac-

cumulation of inflammatory cells in the development of subsequent late-phase reactions and the chronic inflammatory responses of allergic diseases as a chemoattractant.

Mast cells play a major role not only in allergic diseases but also in a number of non-allergic immune reactions, such as fibrotic process, host responses to neoplasms, angiogenesis and tissue remodeling. In the lung, mast cell hyperplasia has been demonstrated in patients with fibrotic lung diseases (47, 48) and in a number of experimental models of pulmonary fibrosis, including exposure to asbestos, silica, and bleomycin (49-51). Since the involvement of monocytes and neutrophils have a provoked role in the pathogenesis of fibrotic lung diseases, leukocyte chemotaxis induced by mast cell chymase evokes leukocyte migration into the disease sites in these disorders. Mast cell chymase may be one of the initial triggers of infiltration of neutrophils and monocytes and may play a role in the inflammatory process in various diseases.

CD13/aminopeptidase N

CD13/aminopeptidase N was reported to have a significant role in the pathogenesis of pulmonary sarcoidosis as a T cell chemoattractant(11).High activity of CD13/aminopeptidase N and lymphocyte chemotactic activity is present in the bronchoalveolar lavage fluid (BALF) from patients with sarcoidosis. The chemotactic activity for lymphocytes is partially decreased by the treatment of the BALF with bestatin, a specific inhibitor for aminopeptidases. Sarcoidosis is a chronic inflammatory disease in which there is a systemic granulomatous process and the lungs are most commonly involved in this disorder. Lung parenchymal lesions in patients with sarcoidosis are characterized by alveolitis associated with the infiltration of CD4+ T lymphocytes and noncaseating granuloma formation (52). Although the triggering agent of sarcoidosis is uncertain, lymphokines released by activated CD4+ T lymphocytes play a pivotal role in the inflammatory process of this disorder. Accordingly, CD13/aminopeptidase N may have a role in T lymphocyte involvement in the sarcoid lung. Recently, we reported that CD13/aminopeptidase N has a significant role in the pathogenesis of joint inflammation in rheumatoid arthritis (manuscript submitted).

CONCLUSION

The migration of neutrophils, mast cells, monocytes and lymphocytes into inflamed lesions is one of the fundamental events of inflammation. During the acute phase of inflammatory and allergic diseases, the predominant migrated cells are neutrophils and mast cells, respectively. Subsequently, during the subacute and chronic phases, monocytes and lymphocytes are mainly migrated. Thus, the chemotactic activity for monocytes and lymphocytes of neutrophil-derived serine proteases and mast cell-derived chymase may have a critical role in chronic inflammation and delayed-type hypersensitivity. A greater understanding of the regulation of the production and action of this enzyme may lead to new insights for the control and treatment of inflammation in allergic and nonallergic diseases.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for General Scientific Research (C) from the Ministry of Education, Science, Sports and Culture and the Ministry of Health and Welfare of Japan.

REFERENCES

1. Baggiolini M, Dewald B, Moser B : Human chemokines : an update. Annu Rev Immunol 15 : 675-705, 1997.
2. Luster AD : Mechanisms of disease : chemokines-chemotactic cytokines that mediate inflammation. N Engl J Med 338, 436-445, 1998.
3. Schall TJ, Bacon KB : Chemokines, leukocyte trafficking, and inflammation. Curr Opin Immunol 6 : 865-873, 1994.
4. Bar-Shavit R, Kahn A, Fenton JW, Wilner GD : Chemotactic response of monocytes to thrombin. J Cell Biol 96 : 282-285, 1983.
5. Bar-Shavit R, Kahn A, Wilner GD, Fenton JW : Monocyte chemotaxis : stimulation by specific exosite region in thrombin. Science (Wash. DC) 220 : 728-731, 1983.
6. Pereira HA, Shafer WM, Pohl J, Martin LE, Spitznagel JK : CAP37, a human neutrophil-derived chemotactic factor with monocyte specific activity. J Clin Invest 85 : 1468-1476, 1990.
7. Chertov O, Michiel DF, Xu L, Wang JM, Tani K, Murphy WJ, Longo DL, Taub DD, Oppenheim

JJ : Identification of defensin-1, defensin-2, and CAP/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. J Biol Chem 271 : 2935-2940, 1996.
8. Chertov O, Ueda H., Xu LL, Tani K, Murphy WJ, Wang, JM, Howard, OMZ, Sayers TJ, Oppenheim JJ : Identification of human neutrophil-derived cathepsin G and azurocidin/CAP37 as chemoattractants for mononuclear cells and neutrophils. J Exp Med 186 : 739-747, 1997.
9. Tani K, Ogushi F, Kido H, Kawano T, Kunori Y, Kamimura T, Cui P, Sone S : Chymase is a potent chemoattractant for human monocytes and neutrophils. J Leukoc Biol 67 : 585-589, 2000.
10. Cui P, Tani K, Kitamura H, Okumura Y, Yano M, Inui D, Tamaki T, Sone S, Kido H : A novel bioactive 31-aminoacid endothelin-1 is a potent chemotactic peptide for human neutrophils and monocytes. J Leukoc Biol 70 : 306-312, 2001.
11. Tani K, Ogushi F, Huang L, Kawano T, Tada H, Hariguchi N, Sone S. : CD13/aminopeptidase N : A novel chemoattractant for T lymphocytes in pulmonary sarcoidosis. Am J Respir Crit Care Med 161 : 1636-1642, 2000.
12. Starkey PM, Barrett AJ : Human cathepsin G : catalytic and immunological properties. Biochem J 155 : 273-278, 1976.
13. Spitznagel JK : Antibiotic proteins of human neutrophils. J Clin Invest 86 : 1381-1386, 1990.
14. Tani K, Murphy WJ, Chertov O, Oppenheim JJ, Wang JM : The neutrophil granule protein cathepsin G activates murine T lymphocytes and up-regulates antigen-specific Ig production in mice. Biochem Biophys Res Comun 282 : 971-976, 2001.
15. Germann T, Bongartz M, Dlugonska H, Hess H, Schmitt E, Kolbe L, Kolsch E, Podlaski FJ, Gately MK, Rude E : Interleukin 12 profoundly up-regulates the synthesis of antigen-specific complement fixing IgG2a, IgG2b and IgG3 antibody subclassed in vivo. Eur J Immunol 25 : 823-829, 1995.
16. Finkelman FD, Katona IM, Mosmann TR, Coffman RL : IFN-γ regulates the isotypes of Ig secreted during *in vivo* humaral immune responses. J Immunol 140 : 1022-1027, 1988.
17. LeGros G, Ben-Sasson SZ, Seder R, Finkelman ED, Paul WE : Generation of interleukin 4 (IL-4)-producing cells *in vivo* and *in vitro* : IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. J Exp Med 172 : 921-929, 1990.
18. Hase-Yamazaki T, Aoki Y : Stimulation of human lymphocytes by cathepsin G. Cell Immunol 160 : 24-32, 1995.
19. Yamazaki T, Aoki Y : Cathepsin G binds to human lymphocytes. J Leukoc Biol 61 : 73-79, 1997.
20. Shafer WM, Martin LE, Spitznagel JK : Cationic antimicrobial proteins isolated from human neutrophil granulocytes in the presence of diisopropyl fluorophospate. Infect Immun 45 : 29-35, 1984.
21. Shafer WM, Martin LE, Spitznagel JK : Cationic antimicrobial proteins isolated from human neutrophil granulocytes in the presence of diisopropyl fluorophosphates. Infect Immun 45 : 29-35, 1984.
22. Flodgaard H, Ostergaard E, Bayne S, Svendsen A, Thomsen J, Engels M, Wollmer A : Covalent structure of two novel neutrophile leucocyte-derived proteins of procine and human origin : neutrophil elastase homologues with strong monocyte and fibroblast chemotactic activities. Eur J Biochem 197 : 535-547, 1991.
23. Welle M : Development, significance, and heterogeneity of mast cells with particular regard to the mast cell-specific proteases chymase and tryptase. J Leukoc Biol 61 : 233-245, 1997.
24. Schechter NM, Brass LF, Lavker RM, Jensen PJ : Reaction of mast cell proteases tryptase and chymase with protease activated receptors (PARs) on keratinocytes and fibroblasts. J Cell Physiol 176 : 365-373, 1998.
25. Mizutani H, Schechter N, Lazarus G, Black RA, Kupper TS : Rapid and specific conversion of precursor interleukin 1β (IL-1β) to an active IL-1 species by human mast cell chymase. J Exp Med 174 : 821-825, 1991.
26. Nadel JA : Role of mast cell and neutrophil proteases in airway secretion. Am Rev Respir Dis 144 : 48-51, 1991.
27. Gordon JR, Burd PR, Galli SJ : Mast cells as a source of multifunctional cytokines. Immnol Today 11 : 458-464, 1990.
28. He S, Walls AF : Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells *in vivo*. Brit J Pharmacol 125 : 1491-1500, 1998.
29. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yasaki Y, Goto K, Masaki T : A novel potent vasocontrictor peptide produced by vascular endothelial cells. Nature 332 : 411-415, 1988.
30. Hanson GC, Anderson KE, Gyllstedt E, Hogesttat ED, Lindberg BF : Hydrolysis of big endothelin-1 by a serine protease in the membrane fraction

of human lung. Regul Pept 68 : 63-69, 1997.
31. Elferink JGR, De Koster BM : Endothelin-induced activation of neutrophil migration. Biohcem Pharmacol 48 : 865-871, 1994.
32. Look AT, Ashmun RA, Shapiro LH, Peiper SC : Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. J Clin Invest 83 : 1299-1307, 1989.
33. Shipp MA, Look T : Hematopoietic differentiation antigens that are membrane-associated enzymes : cutting is the key. Blood 82 : 1052-1070, 1993.
34. Harris CA, Hunte B, Krauss MR, Taylor A, Epstein LB : Induction of leucine aminopeptidase by Interferon-gamma. J Biol Chem 267 : 6865-6869, 1992.
35. Turek JJ, Robinson JP : Leucine aminopeptidase activity by flow cytometry. Methods Cell Biol 41 : 461-467, 1994.
36. Saiki I, Fujii H, Yoneda J, Abe F, Nakajima M, Tsuruo T, Azuma I : Role of aminopeptidase N (CD13) in tumor-cell invasion and extracellular matrix degradation. Int J Cancer 54 : 137-143, 1993.
37. Hansen AS, Noren O, Sjostrom H, Werdelin O : A mouse aminopeptidase N is a marker for antigen-presenting cells and appears to be co-expressed with major histocompatibility complex class II molecules. Eur J Immunol 23 : 2358-2364, 1993.
38. Van Hal PTW, Hopstaken-Broos JPM, Wijkhuijs JM, Te Velde AA, Figdor CG, Hoogsteden HC : Regulation of aminopeptidase-N (CD13) and FcERIIb (CD23) expression by IL-4 depends on the stage of maturation of monocytes/macrophages. J Immunol 149 : 1395-1401, 1992.
39. Lehrer RI, Lichtenstein AK, Ganz T : Antimicrobial and cytotoxic peptides of mammalian cells. Annu Rev Immunol 11 : 105-125, 1993.
40. Weissmann G, Goldstein I, Hoffstein S, Chauvet G, Robineaux R : Yin/Yang modulation of lysosomal enzyme release from polymorphonuclear leukocytes by cyclic nucleotides. Ann N Y Acad Sci 256 : 222-230, 1975.
41. Kudo C, Yamashita T, Araki A, Terashita M, Watanabe T, Atsumi M, Tamura M, Sendo F : Modulation of *in vivo* immune response by selective depletion of neutrophils using monoclonal antibody, RP 3. I. Inhibition by RP-3 treatment of the priming and effector phases of delayed type hypersensitivity to sheep red blood cells in rats. J Immunol 150 : 3728-3738, 1993.
42. Kudo C, Yamashita T, Terashita M, Sendo F : Modulation of *in vivo* immune response by selective depletion of neutrophils using monoclonal antibody, RP-3. II. Inhibition by RP-3 treatment of mononuclear leukocyte recruitment in delayed type hypersensitivity to sheep red blood cells in rats. J Immunol 150 : 3739-3746, 1993.
43. Sekido N, Mukaida N, Harada A, Nakanishi I, Watanabe Y, Matsushima K : Prevention of lung reperfusion injury in rabbits by a monoclonal antibody against interleukin-8. Nature 365 : 654-657, 1993.
44. Larsen CG, Thomsen MK, Gesser B, Thomsen PD, Deleuran BW, Nowak J, Skodt V, Thomsen HK, Deleuran M, Thestrup-Pedersen K. Harada A. Matsushima K, Menne T : The delayed-type hypersensitivity reaction is dependent on IL-8 : Inhibition of a tuberculin skin reaction by an anti-IL-8 monoclonal antibody. J Immunol 155 : 2151-2157, 1995.
45. Taub DD, Anver M, Oppenheim JJ, Long DL, Murphy WJ : T lymphocyte recruitment by interleukin-8 (IL-8) : IL-8-induced degranulation of neutrophils releases potent chemoattractants for human T lymphocytes both *in vitro* and *in vivo*. J Clin Invest 97 : 1931-1941, 1996.
46. Parwaresh, MR, Horny HP, Lennert K : Tissue mast cells in health and disease. Path Res Pract 179 : 439-461, 1985.
47. Kawanami O, Ferrans VJ, Fulmer JD, Crystal RG : Ultrastructure of pulmonary mast cells in patients with fibrotic lung disorders. Lab Invest 40, 717-734, 1979.
48. Hawkins RA, Claman HN, Clark RAF, Steigerwald J : Increased dermal mast cell proliferation in progressive systemic sclerosis : A link in chronic fibrosis? Ann Intern Med 102 : 182-186, 1985.
49. Suzuki N, Horiuchi T, Ohta K, Yamaguchi M, Ueda T, Takizawa H, Hirai K, Shiga J, Ito K, Miyamoto T : Mast cells are essential for the full development of silica-induced pulmonary inflammation : A study with mast-cell deficient mice. Am J Respir Cell Mol Biol 9 : 475-483, 1993.
50. Goto T, Befus D, Low R, Bienenstock J : Mast cell heterogeneity and hyperplasia in bleomycin-induced pulmonary fibrosis of rats. Am Rev Respir Dis 130 : 797-802, 1984.
51. Jordana M : Mast cells and fibrosis : Who' on first ? Am J Respir Cell Mol Biol 8 : 4-8, 1993.
52. Thomas PD, Hunninghake GW : Current concepts of the pathogenesis of sarcoidosis. Am Rev Respir Dis 135 : 747-760, 1987.