Effect of Acute-phase Proteinase Inhibitors on Chemotaxis of Rat Polymorphonuclear Leukocytes *in Vitro**

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Rat serum obtained at 24 h after subcutaneous injection of carrageenin significantly suppressed chemotaxis of rat polymorphonuclear leukocytes (PMNs) *in vitro*. α_2 Acute-phase macroglobulin (α_2 APM), α_1 proteinase inhibitor (α_1 PI) and cysteine-proteinase inhibitors (CPIs) are present at high concentration in the 24-h serum and known as acute-phase reactants in rats. These acute-phase proteinase inhibitors were purified from inflamed rat serum or exudate and their effect on PMN chemotaxis was studied by Boyden's method *in vitro*. α_2 APM (4 mg/ml) significantly suppressed PMN chemotaxis while α_1 M was without effect, though both α_2 APM and α_1 M had a similar anti-proteinase activity. The results suggest that α_2 APM suppressed PMN chemotaxis through the mechanism unrelated to its anti-proteinase activity. On the other hand, α_1 PI (1 and 3 mg/ml) slightly but significantly suppressed PMN chemotaxis, whereas CPI-1 and CPI-2 had no inhibitory effect. These results suggest that α_2 APM and α_1 PI play a role in suppression of PMNs infiltration into the inflammatory site in the late-phase of acute inflammation.

Keywords — proteinase inhibitor; inflammation; chemotaxis; polymorphonuclear leukocyte; α_2 acute-phase macroglobulin; α_1 proteinase inhibitor; acute-phase reactant; acute-phase proteinase inhibitor

Introduction

The acute-phase response in inflammation is associated with the onset of drastic increase in the plasma concentration of some proteins, acute-phase reactants.^{1,2)} These include proteinase inhibitors, proteins with transport or binding function, certain complement components, fibrinogen and other glycoproteins.^{1,2)} Proteinase inhibitors including α_2 APM, α_1 PI (alternate name: α_1 antitrypsin) and CPIs (alternate name: T-kininogens) are representative acute-phase reactants in rats. Changes in plasma concentrations and messenger ribonucleic acid (mRNA) levels of these acute-phase proteinase inhibitors during inflammation have been reported,²⁻¹²⁾ but their roles in inflammation are not clearly known.

It has been reported that proteinase inhibitors such as aprotinin, soybean trypsin inhibitor and ovomucoid suppress various experimental

models of inflammation.¹³⁻¹⁵⁾ In addition it has been suggested that endogenous anti-inflammatory proteins modulate inflammation.¹⁶⁻²⁰⁾ We have reported in previous papers²¹⁻²³⁾ that synthetic proteinase inhibitors including L-1-tosylamide-2-phenylethyl chloromethyl ketone, *N*- α -*p*-tosyl-L-lysine chloromethyl ketone, ϵ -amino-*n*-caproic acid *n*-hexyl ester and leupeptin suppress PMN chemotaxis *in vitro* and PMN migration *in vivo* to the inflammatory site of the carrageenin-induced inflammation in rats. The present studies are a further investigation of anti-inflammatory activity of proteinase inhibitors. We have examined whether acute-phase proteinase inhibitors including α_2 APM, α_1 PI and CPIs, which are endogenous high-molecular-weight inhibitors, suppress PMN chemotaxis *in vitro*.

* Abbreviations used: α_2 APM, α_2 acute-phase macroglobulin; α_1 M, α_1 macroglobulin; α_1 PI, α_1 proteinase inhibitor; CPI(s), cysteine-proteinase inhibitor(s); PMN(s), polymorphonuclear leukocyte(s); ZAS, zymosan-activated rat serum; PAGE, polyacrylamide gel electrophoresis.

Materials and Methods

Preparation of Rat PMNs — PMNs from rat peritoneal cavity were collected by the method of Reed and Tepperman,²⁴⁾ with minor modifications. Briefly, male Wistar rats weighing 350–450 g were injected intraperitoneally with 40 ml of a 1% (w/v) solution of casein in Ca^{2+} -free Krebs-Ringer bicarbonate buffer and the rats were sacrificed 16 h later. The rat abdomen was massaged, the peritoneum was opened, and the exudate fluid was collected and poured through gauze to remove large debris and aggregates. The peritoneal exudate cells were then pelleted by centrifugation at $800 \times g$ for 10 min at 4 °C, washed five times with phosphate-buffered saline, and then suspended to a concentration of 1×10^7 cells per ml of RPMI-1640 medium. The cells obtained were routinely greater than 95% PMNs with viability greater than 97% as assessed by trypan blue dye exclusion.

Chemotaxis Assay — PMN chemotaxis experiment *in vitro* used a multiwell plate as an adaptation of the modified Boyden chamber described previously.²⁵⁾ Briefly, the upper and lower compartments were separated by a polycarbonate membrane (10 μm thickness) with 2 μm pores in diameter. The lower compartment contained 0.42 ml of RPMI-1640 medium containing 1% (v/v) ZAS as a chemoattractant, while the upper one had 0.3 ml of PMN suspension (1×10^7 cells/ml of RPMI-1640 medium) plus/minus the sample to be tested. The Boyden chamber was placed in a humidified incubator at 37 °C with 5% CO_2 –95% air for 60 min, and the number of PMNs migrated into the lower compartment was counted by a Coulter counter.

As an index of PMN chemotaxis, migration rate of PMNs was calculated as follows; (number of PMNs collected from the lower compartment/number of PMNs applied in the upper one) $\times 100$ (%).

Induction of Inflammation — Inflammation was induced according to the procedure of Fukuhara and Tsurufuji²⁶⁾; 5 ml of a 2% (w/v) solution of Seakem 202 carrageenin (Marine Colloid Inc., Springfield, NJ, U.S.A.) was injected into a preformed air-pouch on the back of the

rats.

Blood was obtained by cutting a carotid artery at 24 h after carrageenin injection. The blood was cooled in an ice bath for 2 h and centrifuged at $1000 \times g$ for 30 min at 4 °C. The supernatant (24-h serum) was used for the purification of $\alpha_2\text{APM}$, $\alpha_1\text{M}$ and $\alpha_1\text{PI}$.

The pouch fluid pooled inside the capsule of granulation tissue was collected on day 7 after carrageenin injection, and centrifuged at $100000 \times g$ for 60 min at 4 °C. The supernatant (day-7 exudate) was used for purification of CPIs.

Purification of Proteinase Inhibitors — All purification procedures were carried out at 4 °C. $\alpha_2\text{APM}$ was purified by a modification of the procedure described previously.²⁷⁾ Briefly, 24-h serum (50 ml) was thoroughly dialyzed against 0.05 M Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, 5 mM CaCl_2 and 0.02% NaN_3 , and centrifuged at $9000 \times g$ for 60 min. The supernatant (17 ml) was applied to a Sephadex G-150 column (3.2 $\times$ 82 cm) equilibrated with the same buffer. The gel filtration was repeated 3 times. The first peak (eluted with void volume) from the Sephadex G-150 column was thoroughly dialyzed against 0.05 M Tris-HCl buffer (pH 8.0) containing 0.05 M NaCl and 0.02% NaN_3 . The dialyzed sample was chromatographed on a DEAE-Sephacel column (1.8 $\times$ 27 cm) with a linear gradient of NaCl from 0.05 M to 0.20 M. $\alpha_2\text{APM}$ and $\alpha_1\text{M}$ were separately eluted from the column and the purity of $\alpha_2\text{APM}$ and $\alpha_1\text{M}$ fractions was determined by PAGE.²⁷⁾

For the purification of $\alpha_1\text{PI}$, the albumin fraction obtained from the Sephadex G-150 gel filtration of 24-h serum was used. The albumin fraction which contained $\alpha_1\text{PI}$ was dialyzed against 0.05 M Tris-HCl buffer (pH 8.0) containing 0.02% NaN_3 and applied to Blue Sepharose CL-6B column (2.6 $\times$ 20 cm). Albumin was bound but $\alpha_1\text{PI}$ was not bound to the Blue Sepharose. This unbound fraction was again subjected to the Blue Sepharose column and $\alpha_1\text{PI}$ was bound to the Blue Sepharose because most of the albumin was removed by the first Blue Sepharose column chromatography. $\alpha_1\text{PI}$ was eluted by a linear gradient of NaCl from 0 to 0.5 M. The fractions containing $\alpha_1\text{PI}$ were pooled and dialyzed against 0.02 M Tris-HCl buffer (pH

7.8) containing 0.05 M NaCl, 2 mM CaCl₂ and 0.02% NaN₃. The dialyzed sample was applied to a DEAE-Sephacel column (1.6 × 19 cm). α_1 PI was eluted by a linear gradient of NaCl from 0 to 0.15 M. α_1 PI eluted from the column at about 0.1 M NaCl was pure as determined by PAGE.

CPI-1 and CPI-2 were purified according to the procedure described previously²⁸⁾; CPI-1 and -2 were purified from the day-7 exudate by chromatography on a DEAE-Sephacel column and on a Sephadex G-150 column and finally on a Cm-papain-Sepharose 4B column.

Results

The 24-h serum significantly suppressed PMN chemotaxis *in vitro*, while normal rat serum was without effect (Table I). The results suggest that the 24-h serum contains inhibitory factor(s) toward PMN chemotaxis.

α_2 APM, α_1 PI, CPI-1 and CPI-2 are acute-phase reactants in rats. Their plasma concentra-

tions reach a maximum at 24–48 h after carrageenin injection.^{27,28)} To determine whether these acute-phase proteinase inhibitors suppress PMN chemotaxis, the inhibitors were purified and the effect of the purified inhibitors on PMN chemotaxis was studied *in vitro*. α_2 APM, α_1 M and α_1 PI were purified from 24-h serum. In contrast to α_2 APM and α_1 PI, the exudate concentrations of CPIs were high during the chronic-phase of carrageenin-induced inflammation in rats.²⁸⁾ We, therefore, purified CPI-1 and -2 from day-7 exudate. The purified exudate CPIs were immunologically identical to purified plasma CPIs as determined by double immunodiffusion analysis using rabbit anti-exudate CPI-1 antibody (data not shown). Although the purified α_1 M fraction contained about 10% α_2 APM, the purified acute-phase reactants gave a single band when determined by PAGE (Fig. 1). In addition, a single inhibitor band was found by zymography of each purified acute-phase reactant (data not shown). These results indicate that the purified acute-phase proteinase

TABLE I. Effect of 24-h Serum on PMN Chemotaxis *in Vitro*

	Migration rate (%)		
	Control (medium)	Normal serum	24-h serum
Expt. 1	52.0 ± 1.8	46.1 ± 1.5 (88.7)	47.9 ± 2.0 (92.1)
		46.4 ± 1.5 (89.2)	42.9 ± 1.5 (82.5)
		49.1 ± 1.4 (94.4)	29.5 ± 4.0 (56.7)
Expt. 2	46.0 ± 4.5	45.8 ± 3.0 (99.6)	31.2 ± 0.4 (67.8)
		52.8 ± 1.6 (114.8)	19.3 ± 1.9 (42.0)
		48.8 ± 2.4 (106.1)	50.2 ± 0.4 (109.1)
Expt. 3	53.0 ± 2.4	60.2 ± 1.9 (113.6)	42.1 ± 5.1 (79.4)
		57.8 ± 2.3 (109.1)	38.4 ± 4.1 (72.5)
			52.0 ± 1.8 (98.1)
			40.0 ± 4.4 (75.5)
No. of rats	—	8	10
% of control	—	101.9 ± 3.7	77.6 ± 6.2 a)

The upper compartment of the modified Boyden chamber contained PMN suspension with 10% (v/v) 24-h serum, normal rat serum or RPMI-1640 medium (control). The lower compartment contained 1% (v/v) ZAS. Other conditions are described under Materials and Methods. Each migration rate represents mean ± S.E.M. obtained from 5 or 6 Boyden chambers of each rat serum. The number in each parenthesis indicates percent of the respective control, and the values are summarized as % of control. a) The value is significantly different from that of normal serum; $p < 0.01$.

Fig. 1. PAGE of Purified Acute-phase Proteinase Inhibitors, 24-h Serum (24 hS), Normal Rat Serum (NS) and Day-7 Exudate (Ex)

Purified α_2 APM (APM) and α_1 M (M) were electrophoresed on a 4% polyacrylamide gel, while purified α_1 PI (PI), CPI-1 (C1) and CPI-2 (C2) were electrophoresed on a 11% polyacrylamide gel with 4% stacking gel.

TABLE II. Effect of Acute-phase Proteinase Inhibitors on PMN Chemotaxis *in Vitro*

	Concn. (mg/ml)	No. of chambers	Migration rate (%)
Expt. 1			
Control	—	9	42.7 $\pm$ 2.8
α_2 APM	1	6	34.9 $\pm$ 2.7
	4	6	22.0 $\pm$ 2.8 ^{b)}
α_1 M	1	6	41.7 $\pm$ 4.0
	4	6	40.6 $\pm$ 2.7
Rat albumin	4	6	37.6 $\pm$ 3.4
Expt. 2			
Control	—	5	48.8 $\pm$ 1.9
α_1 PI	1	3	42.6 $\pm$ 6.6
	3	3	39.2 $\pm$ 3.2 ^{a)}
Rat albumin	3	3	47.6 $\pm$ 0.8
Expt. 3			
Control	—	6	62.3 $\pm$ 1.3
α_1 PI	1	6	49.7 $\pm$ 1.7 ^{b)}
Expt. 4			
Control	—	3	32.8 $\pm$ 3.6
CPI-1	1	6	32.2 $\pm$ 4.2
	3	5	30.5 $\pm$ 4.6
Expt. 5			
Control	—	5	56.9 $\pm$ 1.8
CPI-2	1	4	63.4 $\pm$ 3.2
	3	6	61.7 $\pm$ 2.0

Acute-phase proteinase inhibitors or purified rat serum albumin (rat albumin) were applied at indicated concentrations into the upper compartment of the modified Boyden chamber which contained PMN suspension. The lower compartment contained 1% (v/v) ZAS. Migration rates represent means $\pm$ S.E.M. Values are significantly different from the respective control; ^{a)} $p < 0.05$, ^{b)} $p < 0.01$.

inhibitors contain negligible amount of impurity.

The effects of the purified acute-phase proteinase inhibitors on PMN chemotaxis *in vitro* are summarized in Table II. α_2 APM at concentration of 1 mg/ml showed a tendency to suppress PMN chemotaxis and at 4 mg/ml α_2 APM significantly suppressed PMN chemotaxis, whereas α_1 M was without effect. In addition, α_1 PI (1 or 3 mg/ml) slightly but significantly suppressed PMN chemotaxis, though the effect of α_1 PI was variable at concentration of 1 mg/ml. On the other hand, CPI-1 and CPI-2 had no effect on PMN chemotaxis. Effect of purified rat serum albumin on PMN chemotaxis was determined to estimate non-specific protein effect on the assay system. As shown in Table II (expts. 1 and 2) rat albumin (3 and 4 mg/ml) did not affect PMN chemotaxis.

Discussion

Normal rat plasma macroglobulins consist of mainly α_1 M (about 4 mg/ml) together with α_2 APM (about 0.015 mg/ml) as a minor component.²⁹⁾ Plasma concentration of α_2 APM markedly increases and reaches a maximum level (3–10 mg/ml) in the acute-phase of inflammation,^{30–32)} whereas α_1 M concentration maintains a normal level during inflammation.²⁹⁾ In addition we reported that the serum concentration of α_2 APM reached almost the same levels as that of α_1 M at 24 h after carrageenin injection.²⁷⁾ On the basis of these findings, the effect of α_2 APM and α_1 M on PMN chemotaxis *in vitro* was determined by using concentrations of 4 mg and 1 mg/ml of both macroglobulins. α_2 APM, at a concentration of 4 mg/ml, significantly suppressed PMN chemotaxis *in vitro*, while α_1 M was without effect (Table II). In addition, we found that negligible PMN migration occurred when α_2 APM or α_1 M were added to the lower compartment of Boyden chamber at concentrations of 4 or 1 mg/ml (data not shown), suggesting that both the macroglobulins caused neither chemotaxis nor kemokinesis.

In the present studies we purified α_2 APM and α_1 M without performing $(\text{NH}_4)_2\text{SO}_4$ precipitation, because treatment of macroglobulins with ammonium salts causes conformational

changes, resulting in the decrease in their anti-proteinase activity.³³⁾ Both α_2 APM and α_1 M inhibit almost all proteinases by a common mechanism between the two, called “trap” mechanism.³³⁾ Inhibitory activity of purified α_2 APM and α_1 M toward trypsin was similar to each other; IC_{50} of α_2 APM was 5.6 $\mu\text{g/ml}$ and that of α_1 M was 8.4 $\mu\text{g/ml}$. Thus, we used native macroglobulins with high anti-proteinase activity in the present experiments. The results obtained suggest that native α_2 APM suppresses PMN chemotaxis by the mechanism unrelated to its anti-proteinase activity. It has been reported that modified α_2 APM also affects PMN chemotaxis; Gool *et al.*¹⁹⁾ demonstrated that α -macrofetoprotein, a normal fetal rat plasma constituent which is identical with α_2 APM, exerted a strong inhibitory effect on casein-induced PMN chemotaxis *in vitro*, while α_1 M was without effect. They precipitated α -macrofetoprotein and α_1 M with $(\text{NH}_4)_2\text{SO}_4$ in the initial step of purification. It should be pointed out that Gool *et al.* used α_1 M and α -macrofetoprotein with low anti-proteinase activity, and the relationship between the inhibitory effect of both macroglobulins on PMN chemotaxis and that on proteinases is unclear in their report. In addition, Forrester *et al.*³⁴⁾ reported that human α_2 M which had been modified by interaction with proteinases or ammonium sulphate stimulated human neutrophil locomotion *in vitro*. The enhanced neutrophil locomotion by modified α_2 M was kemokinesis but not chemotaxis, while native α_2 M had no effect on neutrophil locomotion. They suggested that the degree of neutrophil locomotory response induced by α_2 M correlated closely with changes in molecular conformation of α_2 M. On the other hand, Goetzl²⁰⁾ demonstrated that native human α_2 M at concentrations of 1–100 $\mu\text{g/ml}$ enhanced random locomotion of human neutrophils and suppressed neutrophil chemotaxis. However, inhibition of neutrophil chemotaxis by low doses of human α_2 M reported by Goetzl²⁰⁾ was not confirmed by Forrester *et al.*³⁴⁾ The discrepancy between the findings of Goetzl and those of Forrester *et al.* remains unexplained. We observed that native α_2 APM at concentrations less than 1 mg/ml did not

affect rat PMN chemotaxis *in vitro*, and the effect of modified α_2 APM on PMN chemotaxis is unclear and must wait a future investigation.

Koj *et al.* ³⁵⁾ demonstrated that plasma concentration of α_1 PI was about 1.7 mg/ml in normal rats and about 2.1 mg/ml at 24 h after subcutaneous injection of turpentine when determined by rocket immunoelectrophoresis. As shown in Table II (expts. 2 and 3) α_1 PI at concentrations of 1 and 3 mg/ml had a weak inhibitory activity on PMN chemotaxis. Negligible migration of PMNs was found when α_1 PI (1 or 3 mg/ml) was added to the lower compartment of Boyden chamber (data not shown), suggesting that α_1 PI caused neither chemotaxis nor chemokinesis. These results suggest that α_1 PI has a weak ability to suppress PMN chemotaxis *in vitro*. On the other hand, CPIs showed no effect on PMN chemotaxis. No inhibition of PMN chemotaxis by albumin, α_1 M and CPIs suggests that α_2 APM and α_1 PI suppress PMN chemotaxis through a specific interaction between both the inhibitors and rat PMNs, though the mechanism is unclear at the present time.

Plasma concentrations of α_2 APM, α_1 PI and CPIs increased after a lag time of 3 h and reached a maximum at 24–48 h after subcutaneous injection of carrageenin in rats (ref. 27 and unpublished data). As shown in Table I, the 24-h serum significantly suppressed PMN chemotaxis. This suppression may be, at least in part, accounted for by high concentrations of α_2 APM and α_1 PI in the 24-h serum. However, we cannot rule out the presence of another factor having inhibitory activity on PMN chemotaxis in the 24-h serum, because the 24-h serum was added to the upper compartment at a concentration of 10% (v/v), in which the concentrations of α_2 APM and α_1 PI may be less than 1 mg/ml.

Prior to the increase in plasma concentrations of acute-phase proteinase inhibitors, PMN migration into the inflammatory site occurs and then diminishes at 24 h after carrageenin injection. On the basis of our results, we think that when plasma concentrations of α_2 APM and α_1 PI reach a maximum level they may participate in the suppression of PMN migration into inflammatory site in the late-phase of acute in-

flammation.

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References

- 1) A. Koj: Metabolic studies of acute-phase proteins, "Pathophysiology of Plasma Protein Metabolism," ed. by G. Mariani, Macmillan Press, London, 1984, pp. 221–248.
- 2) G. Schreiber: Synthesis, processing, and secretion of plasma proteins by the liver and other organs and their regulation, "The Plasma Proteins," vol. 5, ed. by F. W. Putnam, Academic Press, Inc., New York, 1987, pp. 293–363.
- 3) J. Urban, D. Chan and G. Schreiber: A rat serum glycoprotein whose synthesis rate increases greatly during inflammation, *J. Biol. Chem.*, **254**, 10565–10568 (1979).
- 4) A. Koj, A. Dubin, H. Kasperczyk, J. Bereta and A. H. Gordon: Changes in the blood level and affinity to concanavalin A of rat plasma glycoproteins during acute inflammation and hepatoma growth, *Biochem. J.*, **206**, 545–553 (1982).
- 5) G. Schreiber, G. Howlett, M. Nagashima, A. Millership, H. Martin, J. Urban and L. Kotler: The acute phase response of plasma protein synthesis during experimental inflammation, *J. Biol. Chem.*, **257**, 10271–10277 (1982).
- 6) R. Kageyama, N. Kitamura, H. Ohkubo and S. Nakanishi: Differential expression of the multiple forms of rat prekininogen mRNAs after acute inflammation, *J. Biol. Chem.*, **260**, 12060–12064 (1985).
- 7) A. Barlas, H. Okamoto and L. M. Greenbaum: T-Kininogen — the major plasma kininogen in rats adjuvant arthritis, *Biochem. Biophys. Res. Commun.*, **129**, 280–286 (1985).
- 8) W. Sakamoto and K. Yoshikawa: T-Kininogen in rats with carrageenin-induced inflammation, *Biochem. Pharmacol.*, **35**, 4283–4290 (1986).
- 9) H. E. Birch and G. Schreiber: Transcriptional regulation of plasma protein synthesis during inflammation, *J. Biol. Chem.*, **261**, 8077–8080 (1986).
- 10) G. Schreiber, A. R. Aldred, T. Thomas, H. E. Birch, P. W. Dickson, T. Guo-Fen, P. C. Heinrich, W. Northemmann, G. J. Howlett, F. A. de Jong and A. Mitchell: Levels of messenger ribonucleic acids for plasma proteins in rat liver during acute experimental inflammation, *Inflammation*, **10**, 59–66 (1986).

- 11) H. Okamoto, A. Hatta, N. Itoh, Y. Ohashi, K. Arakawa and S. Nakanishi: Acute phase responses of plasma angiotensinogen and T-Kininogen in rats, *Biochem. Pharmacol.*, **36**, 3069—3073 (1987).
- 12) I. Hayashi, S. Oh-ishi, K. Enjyoji, H. Kato and S. Iwanaga: A radioimmunoassay for rat T-Kininogen as an acute phase reactant, *Chem. Pharm. Bull.*, **34**, 3502—3505 (1986).
- 13) Y. G. Hyman and T. L. Vischer: Proteinase inhibitors reduce the reverse passive Arthus reaction, *Agents Actions*, **8**, 532—535 (1978).
- 14) I. Spilberg and C. K. Osterland: Anti-inflammatory effect of the trypsin-kallikrein inhibitor in acute arthritis induced by urate crystals in rabbits, *J. Lab. Clin. Med.*, **76**, 472—479 (1970).
- 15) J. Hladovec, V. Mansfeld and Z. Horakova: Inhibitory action of trypsin and trypsin-inhibitors on experimental inflammation in rats, *Experimentia*, **14**, 146—147 (1958).
- 16) D. A. Lewis, R. B. Capstick and R. Best: The control of rheumatic disease by endogenous protein, *Biochem. Pharmacol.*, **25**, 1435—1436 (1976).
- 17) D. A. Lewis: Endogenous anti-inflammatory proteins, *Biochem. Pharmacol.*, **26**, 693—698 (1977).
- 18) D. A. Lewis: Endogenous anti-inflammatory factors, *Biochem. Pharmacol.*, **33**, 1705—1714 (1984).
- 19) J. van Gool, N. C. J. J. Ladiges and W. Boers: Inhibition of polymorphonuclear leukocyte chemotaxis by α -macroglobulin, an acute-phase reactant of the rat, *Inflammation*, **6**, 127—135 (1982).
- 20) E. J. Goetzel: Modulation of human neutrophil polymorphonuclear leucocyte migration by human plasma alpha-globulin inhibitors and synthetic esterase inhibitors, *Immunology*, **29**, 163—174 (1975).
- 21) H. Nakagawa, K. Shuto, M. Isaji, K. Watanabe and S. Tsurufuji: Proteinase inhibitors suppress the formation of granulation tissue in the carrageenin-induced inflammation in rats, *J. Pharmacobio-Dyn.*, **4**, 429—435 (1981).
- 22) H. Nakagawa, K. Watanabe, K. Shuto and S. Tsurufuji: Anti-inflammatory effect of proteinase inhibitors on carrageenin-induced inflammation in rats, *Biochem. Pharmacol.*, **32**, 1191—1195 (1983).
- 23) H. Nakagawa, K. Watanabe and K. Sato: Inhibitory action of synthetic proteinase inhibitors and substrates on the chemotaxis of rat polymorphonuclear leukocytes *in vitro*, *J. Pharmacobio-Dyn.*, **11**, 674—678 (1988).
- 24) P. W. Reed and J. Tepperman: Phagocytosis-associated metabolism and enzymes in the rat polymorphonuclear leukocyte, *Am. J. Physiol.*, **216**, 223—230 (1969).
- 25) K. Watanabe, H. Nakagawa and S. Tsurufuji: A new simple plastic chemotaxis device of the Boyden chamber type utilizing an immunoassay plate, *Jpn. J. Pharmacol.*, **39**, 102—104 (1985).
- 26) M. Fukuhara and S. Tsurufuji: The effect of locally injected anti-inflammatory drugs on the carrageenin granuloma in rats, *Biochem. Pharmacol.*, **18**, 475—484 (1969).
- 27) H. Nakagawa, K. Watanabe and S. Tsurufuji: Changes in serum and exudate levels of functional macroglobulins and anti-inflammatory effect of α_2 -acute-phase macroglobulin on carrageenin-induced inflammation in rats, *Biochem. Pharmacol.*, **33**, 1181—1186 (1984).
- 28) H. Nakagawa and K. Hirata: Purification of two species of exudate cysteine-proteinase inhibitors that are acute-phase reactants in the carrageenin-induced inflammation in rats, *FEBS Lett.*, **207**, 58—62 (1986).
- 29) A. H. Gordon: The α macroglobulin of rat serum, *Biochem. J.*, **159**, 643—650 (1976).
- 30) H. Hudig and S. Sell: Serum concentration of α -macroglobulin (acute-phase α_2 -macroglobulin), a proteinase inhibitor, in pregnant and neonatal rats and in rats with acute inflammation, *Inflammation*, **3**, 137—148 (1978).
- 31) K. Lonberg-Holm, D. L. Reed, R. C. Roberts, R. R. Herbert, M. C. Hillman and R. M. Kutney: Three high molecular weight protease inhibitors of rat plasma, *J. Biol. Chem.*, **262**, 438—445 (1987).
- 32) F. Ganthier and H. Mouray: Rat α_2 acute-phase macroglobulin, *Biochem. J.*, **159**, 661—665 (1976).
- 33) A. J. Barrett, M. A. Brown and C. A. Sayers: The electrophoretically “slow” and “fast” forms of the α_2 -macroglobulin molecule, *Biochem. J.*, **181**, 401—418 (1979).
- 34) J. V. Forrester, P. C. Wilkinson and J. M. Lackie: Effect of modified α_2 macroglobulin on leucocyte locomotion and chemotaxis, *Immunology*, **50**, 251—259 (1983).
- 35) A. Koj, E. Rogoeczi, C. J. Toews, R. Leveille and J. Gaudie: Synthesis of antithrombin III and alpha-1-anti-trypsin by the perfused rat liver, *Biochem. Biophys. Acta*, **539**, 496—504 (1978).