Inactivation of Human Tumor Cell Pro-urokinase by Granulocyte Elastase

Naohiro Kanayama and Toshihiko Terao

Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31

Supernatant obtained from granulocytes stimulated in the presence of cytochalasin B by the chemotactic peptide N-formyl-norleucyl-leucyl-phenylalanin-norleucyl-tyrosyl-lysine displayed an inhibitory effect on the plasmin-dependent conversion of tumor urokinase-type plasminogen activator proenzyme (pro-uPA) to the active form of uPA. Moreover, the supernatant was also found to inhibit the fibrinolytic activity of human vulva (A431) and breast (MCF7) carcinoma cell lines, which contain large amounts of pro-uPA, by 87% and 96%, respectively. By using eglin C (elastase inhibitor) and a monoclonal antibody to elastase (proteolytic activity blocker of the enzyme), elastase was identified as the key enzyme of the supernatant in these phenomena. Purified elastase converted pro-uPA to an enzymatically inactive molecule composed of two polypeptide chains of Mr = 33,000 and 22,000 linked to each other by a disulfide bond. Elastase-containing granulocytes were identified by immunohistochemistry techniques in the tissues of squamous cell carcinoma and adenocarcinoma of uterus. The cells were found close to the tumor cells and in the stroma surrounding the tumor nests. By immunohistochemical staining, uPA was also found in the tumor cells. Evidently, elastase released by chemotactically activated granulocytes, which are attracted into tumor tissues, may inhibit the conversion of pro-uPA to enzymatically active uPA in the tumor cells.

Key words: Granulocyte elastase — Pro-urokinase — Fibrinolysis — Breast cancer — Uterine cancer

Plasminogen activators are major mediators for pericellular proteolysis. In tissues of breast, prostate, cervix, and colon cancer, urokinase-type plasminogen activator (uPA) is produced and secreted as an enzymatically inactive single-chain proenzyme form (pro-uPA). The increase of uPA in tumor cells has been observed, associated with increased tumor growth and metastatic potential. Pro-uPA may be converted by small amounts of plasmin into the enzymatically active two-chain form uPA (HMW-uPA) which subsequently converts plasminogen into the broad-spectrum serine protease plasmin.

Plasmin degrades the fibrin-fibronectin matrix of the tumor stroma, thus releasing fibrin remnants and cross-linked fibrin-fibronectin compounds.

In solution the naturally occurring plasminogen activator inhibitors PAI-1 and PAI-2 may inactivate uPA, but do not bind to pro-uPA. Here we report that elastase released by chemotactically activated human granulocytes inactivates and degrades tumor cell pro-uPA.

MATERIALS AND METHODS

Reagents were purchased from the sources indicated in parenthesis. Pro-uPA from kidney tumor cell line TCL 598 (specific activity 135,000 units/mg, purity: 98%) (SANDOZ, FRG); purified human granulocyte elastase (purity: 99%) (Protogen, Switzerland); HMW-uPA (Mochida, Tokyo); aprotinin, test thrombin (Behringwerke, FRG); human fibrinogen, human plasmin, and chromogenic substrate S-2444 (KabiVitrum, Sweden); dextran T 500, prestained SDS-PAGE standards (Pharmacia, Sweden); cytochalasin B, rabbit peroxidase-antiperoxidase complex (PAP), and levamisole (Sigma, USA); CHO-Nle-Leu-Phe-Nle-Tyr-Lys (FNLPNTL) and methoxysuccinyl-Ala-Ala-Pro-Val-paranitroanilide (Bachem, FRG); mouse anti-rabbit-IgG, mouse monoclonal antibody to human granulocyte elastase (clone M752), and alkaline phosphatase anti-alkaline phosphatase complex (APAAP) (Dakopatts, Kyoto) mono- and peroxidase-conjugated avidin, and 3,3',5,5'-tetramethylbenzidine (Sigma). Eglin C was a gift from Dr. H. Fritz (FRG).
Inactivation of Pro-urokinase by Elastase

Rabbit-anti human granulocyte elastase was prepared by ourselves.10

Breast cancer cell line MCF7 and vulva cancer cell line A431 cells were supplied by American Type Culture Collection (USA). The cells were grown in RPMI 1640 medium supplemented with l-glutamine and 5% fetal calf serum (FCS) at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Cultures initiated at a density of 5 x 10⁶ cells/ml grew exponentially to about 1 x 10⁸ cells/ml in 3 days. In all the experiments described here 75-cm² tissue culture flasks were inoculated with 30 ml of cell suspension containing 1-3 x 10⁴ cells/ml. All treatments were carried out on exponentially growing cell cultures. Cell viability was assessed by trypan blue exclusion.

Tissue preparation Specimens from patients with uterine cervical and endometrial carcinoma were fixed in buffered saline (PBS).

NaCl was dissolved at room temperature in Tris-HCl, pH 7.3 and NaCl. Enzymatic activity was determined by measuring the change in absorbance at 405 nm. Purified granulocyte elastase (0-0.25 U/ml) served as a 100% activity.

Enzymatic assays of uPA and elastase uPA activity: To determine the effect of elastase on pro-uPA, 50 units of pro-uPA/50 µl of 50 mM Tris-HCl, pH 7.3, was incubated with various concentrations of elastase (0.5-200 mM) for 30 min at 37°C. The mixture will be referred to as elastase-treated pro-uPA. To convert the pro-uPA to uPA, the samples were subsequently incubated with 2.8 mM plasmin (45 min, 37°C). Plasmin action was stopped by the addition of 200 kIU/ml aprotinin.

Samples containing elastase (0-200 nM) in Tris-HCl (in the absence of pro-uPA) with added plasmin served as controls.

The enzymatic activity of uPA was measured with 0.33 mM chromogenic substrate S-2444. Enzyme activity was determined by measuring the change in absorbance at 405 nm. The change of absorbance produced by 50 units pro-uPA/50 µl Tris-HCl which had been treated with 2.8 mM plasmin (45 min, 37°C) was defined as 100% HMW-uPA activity.
the proteolytically degraded forms of uPA as well (Hollrieder, unpublished results). Detection limit: 40 pg of uPA or pro-uPA/ml.

**Stimulation of human peripheral blood granulocytes by N-formyl chemotactic peptide CHO-NLPNTL** Granulocytes were isolated essentially as described. Briefly, 50 ml of fresh human blood obtained from four healthy donors was anticoagulated with 5,000 units of heparin and immediately centrifuged through Ficoll-Hypaque. The cell pellet was resuspended and residual erythrocytes were lysed by using 0.16 M NH₄Cl containing 12 mM NaHCO₃, 0.1 mM EDTA, pH 7.3. Granulocytes were washed with PBS. Granulocytes (5 x 10⁶) in 1 ml of PBS containing 0.1% BSA, 1 mM glucose, 1 mM CaCl₂, and 0.5 mM MgCl₂, pH 7.3, were pretreated with cytochalasin B (5 μl/ml) for 5 min at 37°C. Then 10⁻⁸ M FNLPNTL was added and the cells were incubated for 30 min at 37°C. After low-speed centrifugation (250g, 5 min), the supernatants were collected and then stored at −20°C until measurement.

**Fibrinolytic activity of cancer cells** Ten μl of A431 or MCF7 cells (2 x 10⁶) in PBS, pH 7.3 were preincubated with 40 μl of PBS, supernatant from non-activated granulocytes or supernatant from activated granulocytes (30 min, 37°C). To avoid the influence of myeloperoxidase on cancer cells, 0.25 mM sodium azide was added to the reaction mixture. After low-speed centrifugation (250g), the pellet was collected and resuspended in 10 μl of PBS. Each sample was placed on top of the fibrin gel in the tube (8 h, 37°C). After incubation, lysis volume was calculated as described before. This measurement was performed in triplicate.

To determine the uPA content of A431 or MCF7 cells, 2 X 10⁶ cells were incubated with 0.1% Triton X in Tris buffer, pH 7.3 (12 h, 4°C). After treatment, uPA activity and content were measured as above.

**Immunohistochemical detection of uPA and elastase** Dewaxed paraffin-embedded tissue sections of uterine squamous and adenocarcinoma were processed for the detection of uPA and elastase. The slides were rinsed in 50 mM Tris-HCl containing 125 mM NaCl, pH 8.1 (TBS) and covered with 20% rabbit serum (20 min, 23°C). Subsequently, a 1:50 dilution of monoclonal antibody to uPA (#394) or elastase was added in TBS. After being washed in TBS, the sections were reacted with a 1:50 dilution of Ig rabbit anti mouse Ig in TBS (30 min, 23°C), and again washed with TBS. Then a 1:50 dilution of mouse-APAAP in TBS was added (30 min, 23°C). The sections were washed in TBS, and the alkaline phosphatase-dependent staining was developed with 0.2 mg/ml Naphthol-AS-MX phosphate in combination with 10 mg/ml Fast Red TR in 0.2 M Tris-HCl (pH 8.5) containing 1 mM levamisole to block intrinsic alkaline phosphatase activity (20 min, 23°C). The tissue sections were washed in TBS and water and mounted in glycerol-gelatin. Controls were performed by omitting the first antibody or by replacing the first antibody with irrelevant IgG-antibodies of the relevant species.

**RESULTS**

**Degradation of pro-uPA by purified human granulocyte elastase** To test the effect of elastase on tumor pro-uPA in vitro, elastase was incubated with purified pro-uPA and then the samples were subjected to SDS-PAGE. Elastase degraded pro-uPA (M₉=55,000) into a molecule consisting of two major polypeptide chains of M₉=33,000 and M₉=22,000 connected by a disulfide bond(s) (Fig. 1). In non-reduced condition, this molecule produced by elastase treatment gave a single band of M₉=55,000 (data not shown). Two bands of A chains were observed. The lower band of A chain is thought to be a molecule which lacks the epidermal growth factor (EGF) domain of pro-uPA. The EGF domain of pro-uPA can be split off easily.

**Effect of elastase on pro-uPA activation** The elastase-treated samples were then subjected to plasmin treatment and tested for amidolytic or fibrinolytic activity. The new finding is that elastase treatment of pro-uPA inhibited

![Fig. 1. SDS-PAGE analysis](image-url)
the conversion of pro-uPA by the subsequent addition of plasmin. Elastase has been discovered to digest plasminogen into a smaller fragment (mini-plasminogen). The fragment contains the active site of plasmin and still has the properties of plasmin, especially amidolytic and fibrinolytic activities. Thus, we assume that elastase has no effect on the active site of plasmin.

Therefore, inhibition of the conversion of pro-uPA to active uPA should be the direct effect of elastase on pro-uPA. The potential of pro-uPA to be converted by plasmin into an enzymatically active uPA-molecule (latent activity) decreased with increasing elastase concentration prior to plasmin treatment (Fig. 2). When pro-uPA was treated with elastase first, and then plasmin was added, the enzymatic activity of pro-uPA was completely inhibited at 100 nM elastase. Even at a concentration as low as 0.5 nM elastase, a loss of 35% of the activity was observed. When pro-uPA was incubated with plasmin first, and then elastase (0-200 nM) was added, no loss of pro-uPA activity was detected (data not shown). Samples containing elastase and plasmin did not dissolve a fibrin clot.

Identification of elastase as the functional protease in granulocyte supernatant

When human peripheral blood granulocytes were stimulated in suspension with 10⁻⁸ M chemotactic peptide CHO-NLPNTL, a significant

Fig. 2. Effect of elastase on latent amidolytic and fibrinolytic activity of pro-uPA. For determination of latent amidolytic activity (upper plot) of pro-uPA in the presence of proteases, pro-uPA (50 units) in 50 mM Tris-HCl, pH 7.3, was incubated with elastase (30 min, 37°C), and then 2.8 nM plasmin was added (45 min, 37°C) (△). Fifty units of pro-uPA treated with plasmin in Tris-HCl in the absence of elastase was defined as 100% amidolytic activity. Elastase (75 min, 37°C) (○). For determination of fibrinolytic activity (lower plot) of pro-uPA in the presence of proteases, pro-uPA (50 units) in 50 mM Tris-HCl, pH 7.3, was incubated with elastase (30 min, 37°C), and then applied to the fibrin gels in glass tubes (2 h, 37°C) (△). Elastase only (○) (75 min, 37°C). Lysis caused by 50 units of pro-uPA in Tris-HCl without elastase was defined as 100% fibrinolytic activity.

Table I. Detection of Elastase and uPA in Supernatants Released by Granulocytes Stimulated with the Chemotactic Peptide CHO-NLPNTL

| Reagent                  | Elastase activity (U/ml) | Elastase antigen (µg/ml) | uPA activity (U/ml) | uPA antigen (ng/ml) |
|--------------------------|--------------------------|--------------------------|---------------------|---------------------|
| Buffer                   | ND*                      | 0.02±0.02                | ND                  | 0.29±0.11           |
| 5 µg cytochalasin B/ml   | 0.016±0.01               | 3.2±3.3                  | ND                  | 0.21±0.03           |
| 5 µg cytochalasin B/ml+  | 0.26±0.01               | 62.5±48.7                | ND                  | 0.28±0.07           |
| 10⁻⁸ M CHO-NLPNTL        |                          |                          |                     |                     |

a) Not detected.

Granulocytes (5×10⁶/ml) were preincubated with cytochalasin B (5 µg/ml) and then stimulated with 10⁻⁸ M CHO-NLPNTL as described in the “Materials and Methods” section. The cell supernatants were assayed for elastase activity with the substrate methoxysuccinyl-Ala-Ala-Pro-Val-para-nitroanilide, and for uPA activity with the substrate S-2444. Elastase and uPA antigens were measured by ELISA. Mean values (n=4) are given with the standard deviation.

Note: Human granulocytes contain uPA. Although there is a significant release of enzymatically active granulocyte elastase upon stimulation, no significant release of uPA by granulocytes under the conditions applied was detected by ELISA or enzymatic assay.
amount of enzymatically active elastase was released (Table I). The addition of pro-uPA to supernatant obtained from stimulated granulocytes prevented the conversion of pro-uPA into enzymatically active uPA compared to supernatant obtained from unstimulated cells (Fig. 3). Supernatant of CHO-NLPNTL-stimulated granulocytes was incubated with different concentrations of either eglin C (elastase inhibitor) (0–12 μM) or anti-elastase moAB (blocks activity) (0–15 μM). Figures 4 and 5 show the effect of cell supernatant obtained from stimulated granulocytes on the amidolytic and fibrinolytic activity of pro-uPA in the presence of various amounts of eglin C or moAB with respect to elastase. In the presence of 12 μM eglin C, 96% of the latent enzymatic activity of pro-uPA was preserved (Fig. 4). With 15 μM moAB to elastase, 80% of the latent enzymatic activity was retained (Fig. 5).

**Effect of granulocyte supernatant on fibrinolytic activity of cancer cells** Fibrinolytic activity of A431 and MCF7 cells following various treatments is shown in Fig. 6. A431 and MCF7 cells in PBS buffer dissolved 0.30 ± 0.05 ml and 0.25 ± 0.03 ml, respectively. A431 and MCF7 cells treated with non-activated granulocyte supernatant also lysed 0.30 ± 0.04 and 0.27 ± 0.02, respectively. On the other hand, the lysis volumes of A431 and MCF7 cells incubated with activated granulocyte supernatant were 0.04 ± 0.03 and 0.01 ± 0.02, respectively. Thus, there is a significant difference between the fibrinolytic activity of the cells treated with activated granulocyte supernatant and the others. The inhibition rates of fibrinolytic activity of A431 and MCF7 cells by activated supernatant were 87% and 96%, respectively. Trypan blue staining gave nearly the same value for living cells
Inactivation of Pro-urokinase by Elastase

Fig. 5. Inhibition of elastase activity in granulocyte supernatant by moAB to elastase. Supernatant (60 μl) from granulocytes stimulated with 10^-8 M CHO-NLPNTL in the presence of cytochalasin B (for details see "Materials and Methods" section) were incubated with different concentrations of moAB to elastase (0-15 μM) and then mixed with 50 units of pro-uPA in 60 μl of PBS (30 min, 37°C). Half the volume of the reaction mixture was applied to fibrin clots in tubes (2 h, 37°C) and the lysis volume was measured (lower plot). For the assay of amidolytic activity with substrate S-2444, 2.8 mM plasmin was added to 60 μl aliquots. The reaction was stopped by addition of 200 kIU aprotinin/ml (upper plot). Amidolytic or fibrinolytic activity generated by 50 units of pro-uPA was defined as 100%.

Fig. 6. Effect of granulocyte supernatant on fibrinolytic activity of cancer cells. Epidermoid cancer cells A431 (upper plot) or breast cancer cells MCF7 (lower plot) were incubated with supernatant (40 μl) from granulocytes or tris buffer (30 min, 37°C) containing sodium azide (0.25 mM). Aliquots (60 μl) were applied to a fibrin clot (12 h, 37°C). PMNL: Polymorphonuclear leukocytes.

Localization of elastase and uPA in cancer tissues. Cells containing uPA or elastase were localized in human uterine cervical and endometrial cancer tissues by moAB to the antigens. uPA was localized in the cytoplasm of the cancer cells in formaldehyde-fixed tissues of adenocarcinoma and squamous cell carcinoma (Fig. 7A, 7C). Cancer cells stained homogeneously with the uPA antibody, although differences in staining intensity within the tumors were observed.

Elastase-containing cells were localized in the tumor tissue and also the tumor stroma surrounding the tumor nests (Fig. 7B, 7D). These cells most probably represent granulocytes. The staining pattern of some of these phagocytic cells was irregular, indicating release of elastase into the tumor stroma and tumor cells.

DISCUSSION

Proteins in plasma and tissues, such as fibrinogen, fibronectin, elastin and collagen, have been shown to be substrates for elastase.\textsuperscript{15,16} In this report we demonstrate

Table II. Detection of uPA in Cancer Cell Lines

| Cell line | uPA activity (U/10^6 cells) | uPA antigen (ng/10^6 cells) |
|-----------|-----------------------------|----------------------------|
| A431      | 0.32 ± 0.07                 | 3.1 ± 0.4                   |
| MCF7      | 0.27 ± 0.04                 | 2.8 ± 0.2                   |

After treatment with 0.1% Triton X for A431 and MCF7 (2 × 10^6 cells), uPA activity and antigen were measured by ELISA as described above.
that cancer tissues contain granulocytes which stain for elastase and tumor cells which stain for uPA. In *in vitro* experiments we have shown that purified tumor cell pro-uPA is degraded and inactivated by granulocyte elastase. Dose-dependent inactivation of pro-uPA by elastase was observed for amidolytic and fibrinolytic activity. Treatment of pro-uPA with 100 nM elastase completely prevented the generation of enzymatically active uPA by subsequent plasmin addition to the elastase-treated molecule. However, the inhibitory curves of elastase on uPA activity and fibrinolytic activity were different. This may be explained by the difference of active sites of the enzyme (uPA) in each reaction.

Treatments of pro-uPA with supernatants of chemotactically activated granulocytes markedly depressed the latent enzymatic capacity of pro-uPA *in vitro*. By inhibition experiments with eglin C or an antibody to the active site of elastase, elastase was identified as the key enzyme for this proteolytic activity. Heiple and Ossowski also reported the destructive potential of cell supernatants of stimulated granulocytes on pro-uPA without identifying the proteases involved. The inactivation capacity of the cell supernatants was inhibited by the serine protease inhibitor diisopropylfluorophosphate (DFP). Elastase is inactivated by DFP. Heiple's and our results support the notion that elastase is one of the key enzymes in inacti-
vating pro-uPA released by the cells. In vivo inactivation of pro-uPA by elastase most probably occurs in the extracellular space, because granulocytes are usually activated after penetrating from vessels.

Cancer tissue is a case where phagocytic cells such as granulocytes and pro-uPA-containing tumor cells coexist. Secretd pro-uPA is converted to enzymatically active HMW-uPA by small amounts of plasmin and then tumor-associated fibrinolysis can occur. Degradation products of the tumor stroma (fibrin-fibronectin matrix) may attract phagocytic cells into tumor stroma. Recent studies have demonstrated that even uPA itself is a potent chemotactic factor for granulocytes in vivo in addition to tumor stroma degradation products. However, the functions of granulocytes around tumor cells are little known compared with those of macrophages and lymphocytes. One of the functions attributed to granulocytes accumulated around tumor cells is a cytotoxic effect on the tumor by release of reactive oxidative intermediates upon stimulation. These oxidants are supposed to destroy tumor cells directly. Under our experimental conditions, since we added sodium azide to the reaction mixture, the influence of radical oxygen on the cancer cells could be neglected. Therefore, inhibition of fibrinolytic activity of cancer cells would be mainly due to elastase released from granulocytes.

The immunohistochemical study also supported this hypothesis. Immunohistochemical staining of cancer sections demonstrated that elastase is localized in cells close to uPA-rich cancer cells and in the tumor stroma surrounding the tumor nests. In those regions pro-uPA released by tumor cells could be inactivated by elastase released from granulocytes. Dvorak et al. observed that granulocytes can attach to tumor cells and then elastase-containing granules may be released. The concentration of protease inhibitors in such regions should be lower than in plasma and would not be sufficient to neutralize elastase activity. These findings lead one to speculate that pro-uPA of tumor cells could be inactivated effectively by elastase in tumor tissue. This may have important implications in tumor biology. Inactivation of pro-uPA by elastase may prevent the generation of enzymatically active uPA and thus diminish tumor cell metastasis and invasion.

ACKNOWLEDGMENTS

The technical expertise of Dr. C. Limvarapuss and Mrs. Ohisi is gratefully acknowledged.

(Received April 25, 1990/Accepted July 9, 1990)

REFERENCES

1) Cajot, J. F., Kruithof, E. K. O., Schleunig, W. D., Sordat, B. and Bachmann, F. Plasminogen activators, plasminogen activator inhibitors and procoagulant analysed in twenty human tumor cell lines. Int. J. Cancer, 38, 719–727 (1986).

2) Corti, A., Nolli, M. L., Soffintenti, A. and Cassani, G. Purification and characterization of single-chain urokinase type plasminogen activator (pro-urokinase) from human A431 cells. Thromb. Haemostasis, 56, 219–224 (1986).

3) Stump, D. C., Lijnen, H. R. and Collen, D. Purification and characterization of single-chain urokinase-type plasminogen activator from human-cell culture J. Biol. Chem., 261, 1274–1278 (1986).

4) Markus, G. The relevance of plasminogen activators to neoplastic growth. Enzyme, 40, 158–172 (1988).

5) Gurewicz, V., Pannel, R., Louie, S., Kelley, P., Sudith, A. L. and Greenlee, R. Effective and fibrin-specific clotlysis by a zymogen precursor form of urokinase (pro-urokinase). J. Clin. Invest., 73, 1731–1739 (1984).

6) Dvorak, H. F. Tumors: wounds that do not heal. N. Engl. J. Med., 315, 1650–1659 (1986).

7) Wilhelm, O., Hafter, R., Coppenrath, E., Pflanz, M., Schmitt, M., Babic R., Linke, R., Gossner, W. and Graeff, H. Fibrin-fibronectin compounds in human ovarian tumor ascites and their possible relation to the tumor stroma. Cancer Res., 48, 3507–3514 (1988).

8) Kruithof, E. K. O., Tran-Thang, C., Ransijin, A. and Bachmann, F. Demonstration of a fast-acting inhibitor of plasminogen activators in human plasma. Blood, 64, 907–913 (1984).

9) Kruithof, E. K. O., Vassalli, J. D., Schleunig, W. D., Mattaliano, R. J. and Bachmann, F. Purification and characterization of a plasminogen activator inhibitor from the histiocytic lymphoma cell line U-937. J. Biol. Chem., 261, 11207–11213 (1986).

10) Kanayama, N., Horiuchi, K. and Terao, T. The role of human neutrophil elastase in premature rupture of membranes. Asia Oceania J. Obstet. Gynecol., 14, 389–397 (1988).

11) Laemmli, U. K. Cleavage of structural proteins during assembly of the head of bacteriophage. Nature, 27, 680–685 (1970).

12) Kruithof, E. K. O., Ransijin, A. and Bachmann, F. Influence of detergents on the measurement of the fibrinolytic activity of plasminogen activators. Thromb. Res., 28, 251–260 (1982).

13) Borregaard, N., Heiple, J. M., Simons, E. R. and Clark, R. A. Subcellular localization of the b-cytochrome com-

1001
ponent of the human neutrophil microbicidal oxidase: translocation during activation. *J. Cell Biol.*, **97**, 52–59 (1983).

14) Moroz, L. A. Mini-plasminogen: a mechanism for leukocyte modulation of plasminogen activation urokinase. *Blood*, **58**, 97–104 (1981).

15) Weiz, J. L., Landman, S. L., Crowly, K. A., Birken, S. and Morgan, F. J. Development of an assay for *in vitro* human neutrophil elastase activity. *J. Clin. Invest.*, **78**, 155–162 (1986).

16) Wojtecka-Lukasik, E., Kaczanowska, J., Tomczak, Z., Sopata, I. and Kopec, M. Effect of neutral proteases from human leukocytes on plasma fibronectin. *Thromb. Res.*, **33**, 471–476 (1984).

17) Heiple, J. M. and Ossowski, L. Human neutrophil activator is localized in specific granules and is translocated to the cell surface by exocytosis. *J. Exp. Med.*, **164**, 826–840 (1986).

18) Goldleski, J. J., Lee, R. E. and Leighton, J. Studies on the role of polymorphonuclear leukocytes in neoplastic disease with the chick embryo and Walker carcinoma *256 in vivo and in vitro*. *Cancer Res.*, **30**, 1986–1993 (1970).

19) De Bruin, P. A. F., Griffioen, G., Verspaget, H. W., Verheijen, J. H., Dooijewaard, G., Ingh, H. F. and Lamers, C. B. W. Plasminogen activator profiles in neoplastic tissues of the human colon. *Cancer Res.*, **48**, 4520–4524 (1988).

20) Markus, G., Takita, H., Camiolo, S. M., Corsanti, J. G., Evers, J. L. and Hobika, H. Content and characterization of plasminogen activators in human lung tumors and normal lung tissue. *Cancer Res.*, **40**, 841–848 (1980).

21) Kirchheimer, J. C., Koller, A. and Binder, B. R. Isolation and characterization of plasminogen activators from hyperplastic and malignant prostate tissue. *Biochim. Biophys. Acta*, **797**, 256–265 (1984).

22) Evers, J. L., Patel, J., Madeja, J. M., Scelastaseider, S. L., Hobika, G. H. Camiolo, S. M. and Markus, G. Plasminogen activator activity and composition in human breast cancer. *Cancer Res.*, **42**, 219–226 (1982).

23) Dano, K., Andreassen, P. A., Grondahl-Hansen, J., Kristensen, P., Nielsen, L. S. and Skriver, L. Plasminogen activators, tissue degradation and cancer. *Adv. Cancer Res.*, **44**, 139–266 (1985).

24) Leyer, G. T., Cederholm-Williams, S. A., Gaffney, P. J., Houffbrook, S., Mahmoud, M., Pattison, M. and Burnard, K. G. Urokinase — the enzyme responsible for invasion and metastasis in human breast carcinoma? *Fibrinolysis*, **1**, 237–240 (1987).

25) Schmeller, M. L., Hafter, R., Stemberger, A. and Graeff, H. Plasmin and elastase induced fibrin degradation products and their action on vascular permeability: an experimental study. In “Fibrinogen,” ed. A. Henschen, H. Graeff and F. Lottspeich, pp. 361–364 (1982). Walter de Gruyter, Berlin, New York.

26) Boyle, M. D. P., Chldiedo, V. A., Lawman, J. P., Gee, A. R. and Young, M. Urokinase: a chemotactic factor for polymorphonuclear leukocytes *in vivo*. *J. Immunol.*, **139**, 169–174 (1987).

27) Gudewicz, P. W. and Gilboa, N. Human urokonase-type plasminogen activator stimulates chemotaxis of human neutrophils. *Biochem. Biophys. Res. Commun.*, **147**, 1175–1181 (1987).

28) Gerrad, T. L., Cohen, D. J. and Kaplan, A. M. Human neutrophil-mediated cytotoxicity to tumor cells *J. Natl. Cancer Inst.*, **66**, 483–488 (1981).

29) Clark, R. A. and Klebanoff, S. J. Neutrophil-mediated tumor cell cytotoxicity: role of the peroxidase system. *J. Exp. Med.*, **141**, 1442–1447 (1975).

30) Weiss, S. J., Peppin, G., Oritz, X., Ragsdale, C. and Test, S. T. Oxidative autoactivation of latent collagenase by human neutrophils. *Science*, **227**, 747–749 (1985).

31) Dvorak, A. M., Connell, A. B., Proppe, K. and Dvorak, H. F. Immunologic rejection of mammary adenocarcinoma (TA3-St) in C57BL/6 mice: participation of neutrophils and activated macrophages with fibrin formation. *J. Immunol.*, **120**, 1240–1248 (1978).

1002