Experimental Lung Research

ISSN: 0190-2148 (Print) 1521-0499 (Online) Journal homepage: https://www.tandfonline.com/loi/ielu20

Alveolar Macrophage Plasminogen Activator

Mark Schuyler & Walter Forman

To cite this article: Mark Schuyler & Walter Forman (1984) Alveolar Macrophage Plasminogen Activator, Experimental Lung Research, 6:2, 159-169, DOI: 10.3109/01902148409087903

To link to this article: https://doi.org/10.3109/01902148409087903

Published online: 02 Jul 2009.

Alveolar Macrophage Plasminogen Activator*

Mark Schuyler and Walter Forman

ABSTRACT: *Plasminogen activator is a neutral serine protease secreted by many different cells, including activated peritoneal macrophages, which can mediate both inflammation and fibrinolysis and perhaps cytolysis of tumor cells. Secretion of plasminogen activator by rabbit alveolar macrophages derived from normal animals and rabbits pretreated with bacillus Calmette–Guérin (BCG) to activate these macrophages was examined. Plasminogen activator was secreted into media of cultured alveolar macrophages, but was not present within the cells. Secretion, which was dependent upon the presence of viable cells, could be blocked by protein synthesis inhibitors and enhanced by concanavalin A and phorbol myristate acetate. The inhibition profile of rabbit alveolar macrophage plasminogen activator is consistent with that of a serine protease. Plasminogen activator is present in two forms with molecular weights of 28,000 and 45,000. Alveolar macrophage plasminogen activator was secreted in cultures from most rabbits (17 of 23) pretreated with BCG, but rarely in those from normal animals (2 of 14). Lavage fluids from many rabbits contained viable Bordetella bronchiseptica, but the presence of this organism showed no correlation with secretion of plasminogen activator. Rabbit alveolar macrophages secrete a plasminogen activator similar to that secreted by mouse peritoneal macrophages as described previously. Secretion is enhanced by activation of alveolar macrophage populations.*

INTRODUCTION

Plasminogen activator, a neutral serine protease, is secreted by many different cell types and is thought to be important in cell migration, malignant cell transformation, tissue remodeling, and cytolysis of tumor cells [1–5]. Plasmin, produced by the action of plasminogen activator on the serum zymogen plasminogen, is the main source of serum fibrinolytic activity and can induce inflammation by activating the complement and kinin systems [2–6]. Fibrin deposition and inflammation are present within the alveoli of the lung in a variety of pulmonary disorders, including adult repiratory distress syndrome. Bronchoalveolar lavage fluid contains plasminogen activator activity [7]. Although there are many potential sources for this activity (for example, endothelial cells, polymorphonuclear neutrophils, and fibroblasts), alveolar macrophages are the most common cells in bronchoalveolar lavage fluids and certainly a possible source of this enzyme. Alveolar macrophage plasminogen activator could be important in resolution of fibrin clots, promotion of inflammation, or defense against malignant cells in the lung.

*A preliminary report of these data was presented at the Annual Meeting of the Central Society for Clinical Research, November 4–6, 1981, Chicago (Clin Res 29:775A, 1981).

From the Department of Medicine, Cleveland Veterans Administration Medical Center and Case Western Reserve University, Cleveland, Ohio.

Address correspondence and requests for reprints to: Mark Schuyler, M.D., Cleveland VA Medical Center, 10701 East Boulevard, Cleveland, OH 44106.

Received 26 June 1983; accepted 29 August 1983.

Results of previous examinations of the ability of alveolar macrophages to secrete plasminogen activator have been contradictory, with reports of its presence [8–10] and absence [11]. This discrepancy could be caused by differences in the state of activation of macrophages. Activated, but not resident, mouse peritoneal macrophages are known to secrete plasminogen activator [12]. Unlike resident peritoneal macrophages, alveolar macrophages are continually exposed to particles that could alter their population to resemble the activated peritoneal macrophage population, thus eliminating differences between resident and activated alveolar macrophage populations. Therefore, we examined the ability of the alveolar macrophage to secrete plasminogen activator, determined the characteristics of secretion and of the enzyme, and compared secretion of plasminogen activator by alveolar macrophage populations lavaged from normal rabbits to that from rabbits treated with bacillus Calmette–Guérin (BCG), an agent known to activate alveolar macrophage populations [13].

MATERIALS AND METHODS

Animals

New Zealand white rabbits (A. Gutman, Chardon, OH) weighing 2.5–3 kg were individually housed and allowed food and water ad libitum. Mice (C57B1/6); National Cancer Institute, Fort Deterick, MD) were housed five to a cage and allowed food and water ad libitum.

Alveolar Macrophages

Activated alveolar macrophages were obtained from rabbits sensitized to mycobacteria by injection of 0.4 ml of an emulsion of complete Freund's adjuvant (Difco Laboratories, Inc., Detroit, MI) and normal saline into two footpads, then treated intravenously with 100 μg of killed BCG (strain L-1011, Trudeau Institute, Saranac Lake, NY) mixed with oil (Marcol 52, Exxon Corporation, Houston, TX) 2 weeks thereafter. Alveolar macrophages were harvested 14–21 days after administration of BCG.

Normal rabbits were either injected intravenously with an equal volume of normal saline or used with no experimental manipulation. There was no difference between results from normal animals with and without injection of normal saline.

Alveolar macrophages were obtained by lavaging rabbit lungs aseptically with eight 60-ml washes of sterile normal saline solution (0.9% NaCl). The effluent was filtered through sterile gauze to remove mucus and centrifuged, and cells were washed with sterile normal saline three times. The cells were counted on a hemocytometer, viability was determined by trypan blue dye exclusion, and cell type was determined by incubation with neutral red dye [15]. All lavage fluids were cultured for the presence of bacteria on blood agar.

Alveolar macrophages were allowed to attach to glass and exposed to sheep red blood cells coated with immunoglobulin G. The mean number of ingested sheep red blood cells per macrophage is the phagocytic index [15].

Peritoneal Macrophages

Thioglycolate-induced peritoneal macrophages from mice were harvested by lavage with Hank's balanced salt solution with 10 units/ml solium heparin 4 days after intraperitoneal injection of 0.5 ml of thioglycolate broth (Difco).

Macrophage Cultures

Macrophages (both alveolar and peritoneal) were suspended in either Dulbecco's minimum essential medium (DMEM, Gibco, Grand Island, NY) plus 15% acid-treated fetal calf serum [5] or Neuman–Tytell serumless medium (Gibco) plus penicillin (100 units/ml) and strepyomycin (100 μg/ml) at 5×10^5 cells/ml. The type of medium did not affect the results. Cells (5×10^6) were allowed to adhere overnight in 100-mm plastic petri dishes (Falcon 3003, Oxnard, CA) in 5% CO_2 humidified atmosphere. The plates were washed three times with DMEM plus 0.02% lactalbumin hydrolysate (Gibco) and penicillin–streptomycin, and 25 ml of fresh DMEM/lactalbumin hydrolysate/penicillin–streptomycin was added to each plate. Thereafter, the conditioned medium was replaced every 48 hr.

The following substances were added to some alveolar macrophage cultures from rabbits treated with BCG at the indicated final concentrations: cycloheximide, 0.5 μg/ml (Sigma Corporation, St. Louis, MO); puromycin, 0.5 μg/ml (Sigma); and phorbol myristate acetate, 0.01 μg/ml (Consolidated Midland, Brewster, NY). Some cultures were heated (56°C, 30 min) to kill cells.

Cell number and viability were evaluated by counting and determining trypan blue dye exclusion of cells mechanically removed from petri dishes with a rubber policeman. Cell counts were done on a hemocytometer.

Medium plates were treated identically, but had no macrophages.

Conditioned Medium

After each 48-hr period (that is day 2–3, day 4–5, and so on), the conditioned medium was centrifuged (1000G, 15 min, 4°C) to remove cellular debris, dialyzed in 0.1% bovine serum albumin-coated dialysis bags (molecular weight cutoff 10,000; Fisher Scientific, Warrensville Heights, OH) against 10 mM Tris buffer, pH7.5, with 2 mM $CaCl_2$, lyophilized, and reconstituted to 1% of the original volume.

Cell Sonicates

Macrophages (5×10^6 or those removed from one plate at days 1, 3, and 5) were suspended in 10 mM Tris buffer, pH 7.5, sonicated for 90 sec (setting, 2.5 Heat Systems, New York, NY) dialyzed, lyophilized, and reconstituted to 10% of the original volume.

Plasminogen Activator Assay

A modification of the fibrin–agar method of Astrup was used [16]. Fibrinogen (grade L, AB Kabi, Stockholm, Sweden) was mixed with 1.25% agarose (Sigma), allowed to adhere to slides, and exposed to a thrombin bath (1000 units/ml; Parke-Davis, Morris Plains, NJ) for 60 sec to convert fibrinogen to fibrin. The final concentration of fibrin was 2 mg/ml. All slides were used within 24 hr. Plasminogen-free thrombin and fibrinogen were prepared by passing thrombin and fibrinogen (1000 units/ml and 10 mg/ml, respectively) through lysine–Sepharose 4-B columns (Pharmacia Corporation, Piscataway, NJ). All plasminogen-free fibrin–agar plates displayed lysis with plasmin (AB Kabi) and urokinase (Calbiochem, San Diego, CA) and no lysis with streptokinase (ICN, Cleveland, OH).

Samples (10 μl) of conditioned medium and cell sonicates were placed in holes cut in agar and incubated at 37°C in a humidified atmosphere for 24 hr. A set

of streptokinase standards (1, 10, 25, 50, and 100 units/ml) tested simultaneously was used to construct a standard curve relating lysis area to units of streptokinase. Results were expressed as streptokinase units. Samples that caused lysis of the plasminogen-rich fibrin–agar gel all failed to cause lysis of plasminogen-free fibrin plates.

Inhibitor Profile

The following substances were dissolved in 0.05 M phosphate–0.15 M NaCl buffer, pH 7.5: 0.05 M ethylenediaminetetraacetic acid (EDTA); 0.01 M phenylmethylsulfonylfluoride (PMSF); and 0.01 M dithiothreitol (DTT). Equal volumes of inhibitors and enzymes were incubated for 40 min at room temperature and dialyzed against buffer in 0.1% bovine serum albumin-coated dialysis tubing. Activity was related to activity of enzymes incubated with buffer rather than inhibitor.

Polyacrylamide Electrophoresis

The methodology of Granelli-Piperno and Reich [17] was used. Sodium dodecyl sulfate–7% polyacrylamide gels (Sigma) were prepared [18]. Concentrated conditioned medium (100 μl) or a set of molecular weight standards (human serum albumin, ovalbumin, pepsin, and cytochrome *c*; (Sigma) was electrophoresed (3.5 mA/tube) for 18 hr. The gels were washed for 4 hr in 2% Triton-X (Sigma), then in double-distilled water and sliced in half. One-half was stained with Coomassie blue dye and the second half applied to a fibrin–agar plate. The molecular weight of the substance that caused localized lysis of the fibrin was determined by comparing its mobility to that of standards.

Statistical differences were analyzed by the unpaired *t* test and the χ^2 test [19].

RESULTS

Intravenous injection of BCG resulted in a great increase in the number of cells that could be lavaged from rabbit lungs. As indicated in Table 1, animals treated with BCG showed a greater than four fold increase in number of lavaged

Table 1 Bronchoaveolar lavage

Rabbits	Number of cells ($\times 10^{-6}$)	Viability[a] (%)	Macrophages[b] (%)	Phagocytic index[c]
Normal (n = 14)	72 ± 14[d]	86.9 ± 2.5	89.9 ± 1.7	0.33 ± 0.14
BCG (n = 23)[e]	298 ± 40	89.2 ± 1.8	82.2 ± 1.3	3.84 ± 0.7
P value (t test)	<0.01	NS	<0.05	<0.01

Abbreviations: BCG = bacillus Calmette–Guérin; NS = not significant.

[a]Determined by trypan blue dye exclusion.

[b]Determined by uptake of neutral red dye.

[c]Mean number of immunoglobulin G-coated sheep red blood cells phagocytosed per macrophage.

[d]All values represent mean ± standard error of mean.

[e]Rabbits were sensitized to mycobacteria by footpad injection of complete Freund's adjuvant, followed 2 weeks later by intravenous administration of BCG. Animals were killed 2–3 weeks thereafter.

cells. Viability was the same in both populations. The percentage of macrophages (determined by uptake of neutral red dye) was slightly lower in the group treated with BCG. The alveolar macrophages were activated, as indicated by an increase in the phagocytic index [15].

Plasminogen activator activity was detected in the conditioned medium of day 2–3 alveolar macrophage cultures from 17 of 22 animals treated with BCG, opposed to 2 of 14 normal animals ($P < 0.001$, χ^2 test). Cultures of alveolar macrophages from rabbits treated with BCG produced 35–30,000 streptokinase units/10^6 cells. Cultures from the two normal rabbits produced 20 to 100 streptokinase units/ml, respectively. Many (22 of 37) of the rabbits had chronic pulmonary infection, as demonstrated by bronchoalveolar lavage cultures positive for *Bordetella bronchiseptica*. There was no correlation ($P > 0.10$) between the presence of *Bordetella* in lavage fluid and plasminogen activator in conditioned medium from cultured alveolar macrophages.

We examined the influence of protein synthesis inhibitors and heat killing of the cells on the occurence of plasminogen activator activity in conditioned medium from alveolar macrophages derived from rabbits treated with BCG. As indicated in Table 2, heating the culture plates sufficiently to kill 85% of cells abolished production of plasminogen activator. Similarly, both cycloheximide and puromycin greatly reduced production of plasminogen activator, but did not significantly change the number of viable cells per culture. The addition of cycloheximide and puromycin to conditioned medium after removal from the culture dishes did not increase or decrease plasminogen activator activity using our assay system.

The effects of Concanavalin A (Con A) and phorbol myristate acetate, (two agents reported to increase secretion of marcophage neutral proteases [8, 20]), were evaluated. The results (Table 3) indicated that both Con A and phorbol myristate acetate greatly increased secretion of rabbit alveolar macrophage plasminogen activator, although the higher concentration of Con A had an adverse effect on viability. Nether agent affected the plasminogen activator assay, as the addition of these substances to culture supernatants both with and without plasminogen activator activity did not alter the amount of lysis.

The kinetics of secretion of alveolar macrophage plasminogen activator is illustrated by a representative experiment (Table 4). Secretion was minimal at day

Table 2 Inhibition of alveolar macrophage plasminogen activator production[a]

Experiment no.	No. of cells	Cycloheximide (0.5 μg/ml)	Puromycin (0.5 μg/ml)	Heat killed[b]
1	30,000	0	ND	ND
2	2000	0	ND	ND
3	35	0	0	0
4	260	0	0	0
Viable cells[c]		69	86	15

Abbreviation: ND = not determined.

[a]Results expressed as ratio of plasminogen activator activity (streptokinase units per 10^6 cells) to control alveolar macrophage cultures.

[b]Thirty minutes at 56°C.

[c]Number of viable cells per culture, expressed as percentage of control alveolar macrophage cultures. Alveolar macrophages were derived from rabbits treated with bacillus Calmette–Guérin.

Table 3 Induction of alveolar macrophage plasminogen activator secretion[a]

Experiment no.	Con A 1.0 μg/ml	Con A 10.0 μg/ml	PHA 0.01 μg/ml
1	4.5	ND	ND
2	4.0	ND	ND
3	ND	ND	4.7
4	4.7	30.8	3.6
5	3.9	48.5	ND
Viable cells[b]	77	39	89

Abbreviations: Con A = concanavalin A; PMA = phorbol myristare acetate; ND = not determined.

[a]Results expressed as ratio of plasminogen activator activity (streptokinase units per 10^6 cells) to control aveolar macrophage cultures.

[b]Number of viable cells per culture, expressed as percentage of control alveolar macrophage cultures. Alveolar macrophages were derived from animals treated with bacillus Calmette–Guérin.

2–3 after initiation of culture, increased greatly at day 4–5, and then decreased.

The activity of rabbit alveolar macrophage plasminogen activator was not inhibited by EDTA; however, it was inhibited totally by PMSF and partially by DTT (Table 5). In contrast, streptokinase was not inhibited by EDTA, PMSF, or DTT, and urokinase was inhibited by PMSF, but not by EDTA or DTT. Therefore, rabbit alveolar macrophage plasminogen activator acts as a serine protease that is not dependent upon metallic ions and contains sulfhydryl groups.

We determined the location of plasminogen activator on polyacrylamide gels by examining activity (lysis) rather than protein staining, because conditioned medium has small amounts of plasminogen activator and preliminary experiments with streptokinase indicated that the functional assay could detect 50 streptokinase units, whereas the protein stain could not detect less than 1000 units. Zones of lysis appeared in two locations (Fig. 1), corresponding to molecular weights of 28,000 and 45,000. The activity of the smaller molecular weight species appeared to be greater, as the area of lysis around its location was larger. Protein stains of the gels showed multiple bands corresponding to molecular weights of 12,000–60,000. The same bands were present in conditioned medium from cultures with and without macrophages, however, and there were no protein bands in the locations corresponding to the zones of lysis in conditioned medium from macrophage cultures. Using the same techniques of mac-

Table 4 Time course of alveolar macrophage plasminogen activator secretion[a]

Days 2–3	Days 4–5	Days 6–7
50	2460	290

[a]Results expressed as streptokinase units per 10^6 cells. Alveolar macrophages were derived from rabbits treated with bacillus Calmette–Guérin.

Table 5 Inhibition profile of rabbit alveolar macrophage plasminogen activator[a]

Profile	EDTA	PMSF	DTT
Rabbit alveolar macrophage plasminogen activator	100	13	66
Streptokinase (100 U/ml)	121	100	113
Urokinase (100 U/ml)	95	11	96

Abbreviations: EDTA = ethylenediaminetetraacetic acid; PMSF = phenylmethylsulfonylfluoride; DTT = dithiothreitol.

[a]Mean of four experiments. Results expressed as percentage of enzyme incubated with buffer.

rophage culture, collection and concentration of day 2–3 culture fluid, and estimation of molecular weight using lysis on gels, plasminogen activator from thioglycolate-induced mouse peritoneal macrophages exhibited a molecular weight of 24,000 and urokinase molecular weights of 43,000 and 60,000. These results are similar to those reported by Granelli-Piperno and Reich [17] using a similiar technique.

Sonicates from alveolar macrophages from days 0 and 1 of cultures caused lysis of both plasminogen-containing and plasminogen-free fibrin–agar plates in five experiments. The area of lysis on the plasminogen-free fibrin was larger than that on the plasminogen-containing fibrin. Thus, the lysis could be attributed not to plasminogen activator, but to nonspecific proteolysis.

DISCUSSION

Plasminogen activator is secreted by rabbit alveolar macrophages in culture, as determined by plasminogen-dependent lysis of fibrin in agar. We did not find plasminogen activator in cell sonicates at the beginning or during the course of cell culture, but did find plasminogen-independent fibrin lysis, presumably because of the presence of other proteolytic enzymes. Secretion of plasminogen activator was dependent upon the presence of viable cells and could be blocked by protein synthesis inhibitors and stimulated by Con A and phorbol myristate acetate. Secretion was low at the beginning of culture, peaked at day 4–5, and diminished thereafter. The secreted plasminogen activator was inhibited by PMSF, but not by EDTA, consistent with a serine protease. Activity of plasminogen activator on polyacrylamide gel electrophoresis was present at locations corresponding to molecular weights of 28,000 and 45,000. Therefore, rabbit alveolar macrophage plasminogen activator resembles mouse peritoneal macrophage plasminogen activator, which is also predominantly secreted, is a serine protease, is inducible by Con A and phorbol myristate acetate, and has molecular weights of 28,000 and 48,000 [10, 17, 20]. Despite constant exposure to environmental particles and many similarities to activated rather than resident peritoneal macrophage populations [21], alveolar macrophage populations also require activation to become capable of secreting plasminogen activator. In this regard, alveolar macrophages resemble peritoneal macrophages.

Concanavalin A can induce secretion of plasminogen activator either by direct effects of macrophages [20] or through production of a lymphokine, an inducer of plasminogen activator, from lymphocytes exposed to Con A [22, 23]. As our bronchoalveolar population included both lymphocytes and macrophages, we cannot distinguish between direct and indirect effects of Con A on macrophages. The kinetics of production of plasminogen activator by alveolar macrophages is somewhat different from that of peritoneal macrophages in having a lag period

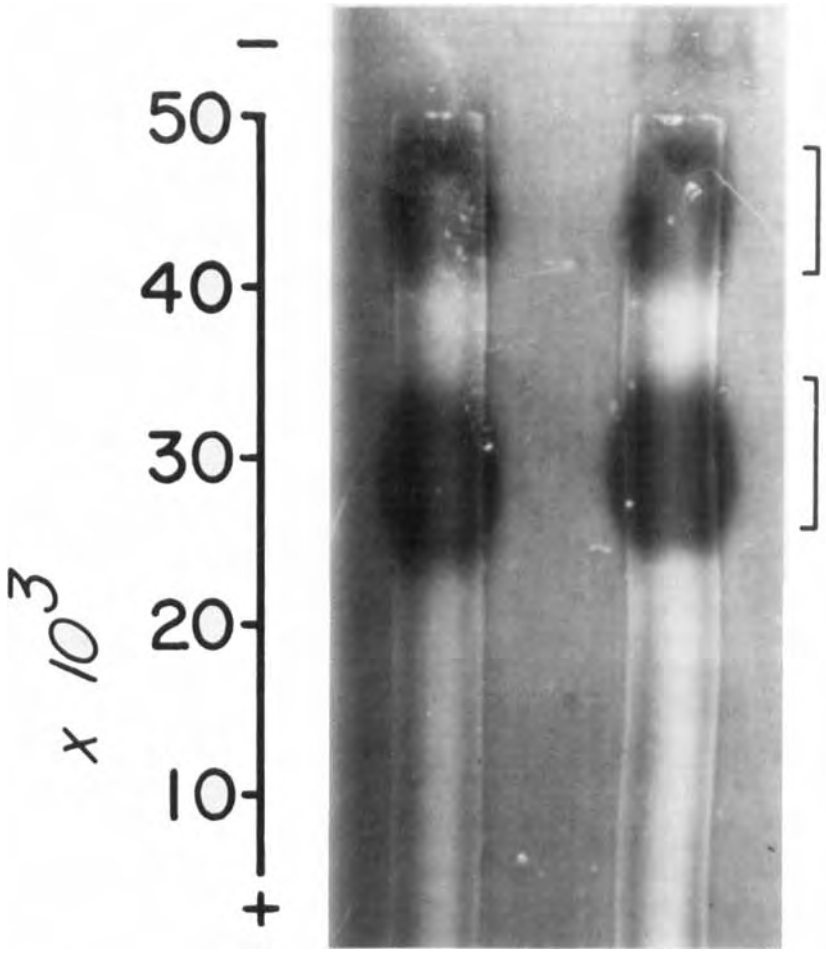

Figure 1 Identification of plasminogen activator after solium dodecyl sulfate–polyacrylamide gel electrophoresis conditioned media. Conditioned media (100μl) were electrophoresed on sodium dodecyl sulfate–7% polyacrylamide gel, and the gel was processed for detection of fibrinolytic activity in the presence of plasminogen. The fibrin–agar indicator gel is shown.

of 3 days before peak production. This lag could be due to remnants of serum protease inhibitors in the acid-treated fetal calf serum or to inactive forms of plasminogen activator, which require proteolytic enzyme treatment to become evident. In fact, Bernik et al. reported the presence of latent plasminogen activator in lung cell cultures [24]. Trypsin treatment of conditioned medium activated plasminogen activator, especially that from newly initiated cultures. A similar phenomenon could have caused our results.

The presence of plasminogen activator in conditioned medium was dependent upon pretreatment of rabbits with BCG, an agent that activates alveolar macrophage populations [13], but was not related to colonization of rabbit lungs with *Bordetella bronchiseptica*, which alters certain biochemical functions of alveolar macrophages [25].

Plasminogen activator is secreted by many different cell types, including fi-

broblasts [26], neuronal cells [1], transformed cells [27, 28], hemopoietic precursor cells [3], endothelial cells [29], kidney cells [30], islet of Langerhans cells [31], lymphocytes [5], and macrophages [4, 8, 12, 20]. Evidence is conflicting, however, regarding the ability of alveolar macrophages to secrete plasminogen activator. Lin and Gordon reported secretion in normal mouse alveolar macrophages [8]; Keller et al. found secretion possible in monkey alveolar macrophages [9]; Wilson et al. reported secretion in rabbit alveolar macrophages from both normal animals and those with experimental hypersensitivity pneumonitis [10]; and Birnbaum and Slauson found plasminogen activator in cell-free bronchoalveolar lavage from normal hamsters [7]. In contrast, Bang and coworkers found that rabbit alveolar macrophages elicited by intravenous complete Freund's adjuvant did not secrete this enzyme [11].

There are several possible reasons for the discordance between our results (activated, rather than normal, rabbit alveolar macrophages secrete plasminogen activator and those of Bang et al. (activated rabbit alveolar macrophages do not secrete plasminogen activator), Lin and Gordon (normal mouse alveolar macrophages secrete plasminogen activator), and Wilson et al. (alveolar macrophages from rabbits with hypersensitivity pneumonitis secrete more plasminogen activator than those from normal rabbits). Bang et al. [11] used a synthetic substrate, L-pyroglu-Gly-Arg-pNa HCl, which has not been shown to detect small amounts of cellular plasminogen activator. Lin and Gordon and Wilson et al. used an assay for plasminogen activator, more sensitive than the fibrin plate method [8, 32] used by us, that measures release of radioactivity from labeled fibrin. We may have been able to detect a small amount of plasminogen activator secretion by normal alveolar macrophages using a more sensitive system. With the fibrin–agar assay system, however, there was a clear dependence of alveolar macrophage plasminogen activator secretion upon activation of the alveolar macrophage population. In addition, there are species differences between mice and rabbits. Compared to activated rabbit macrophages, mouse macrophages exhibit exaggerated attributes of activation (that is, complement-mediated phagocytosis) [15]. Therefore, either species or methodologic differences could have caused our results to differ from those in previous reports.

Many different functions have been attributed to plasminogen activator, including lymphocyte blastogenesis [33], production of the cutaneous basophil hypersensitivity reaction [34], cell migration, induction of the inflammatory response through interaction with the kinin and complement systems [2, 6], and lysis of tumor cells by activated macrophages [4] and natural killer lymphocytes [5]. In the lung, production of alveolar macrophage plasminogen activator associated with activation of alveolar macrophages could be a mediator of intraalveolar inflammation and/or allow dissolution of fibrin clots during resolution of inflammation. Cigarette smoking greatly increases the number of alveolar macrophages that are activated in some respects [21] and is associated with distinctive peribronchiolar inflammation and accumulation of alveolar macrophages at the level of the terminal bronchiole [35]. Alveolar macrophage plasminogen activator could be important in initiation and perpetuation of this lesion. Further study of production of alveolar macrophage plasminogen activator in rabbits subjected to experimental manipulation could aid in understanding of its role in response to environmental stimuli.

This study was supported by NIOSH Grant OH-00992 and the Medical Research Service, Cleveland Veterans Administration Medical Center.

REFERENCES

1. Krystosek A, Seeds N: Plasminogen activator release at the neuronal growth cone. Science 213:1532–1534, 1981.
2. Christman J, Silverstein S, Acs G: Plasminogen activators in proteinases. In: Barrett AJ, ed., Mammalian Cells and Tissues. New York, North–Holland Biomedical Press, 1977, pp. 91–149.
3. Valinsky J, Reich E, Douarin N: Plasminogen activator in the bursa of Fabricus: Correlation with the morphogenetic remodeling and cell migrations. Cell 25:471–476, 1981.
4. Adams DO: Effect of mechanism of cytolytically activated macrophages. I. Secretion of neutral protease and effect of protease inhibitors. J Immunol 124:286–292, 1980.
5. Roder J, Kiessling R, Biberfeld P, Anderson E: Target-effect or interaction of the natural killer (NK) cell system. II. The isolation of NK cells and studies on the mechanism of killing. J Immunol 121:2509–2517, 1978.
6. Ratnoff O, Naff G: The conversion of C'1 esterase by plasmin and trypsin, J Exp Med 125:337–350, 1967.
7. Birnbaum S, Slauson D: Pulmonary fibrinolytic activity: Development of a sensitive radiochromatographic assay. Isr J Med Sci 14:448–454, 1978.
8. Lin H-S, Gordon S: Secretion of plasminogen activator by bone marrow-derived mononuclear phagocyes and its enhancement by colony-stimulating factor. J Exp Med 150:231–245, 1979.
9. Keller R, Calvanico N, Stevens J: Hypersensitivity pneumonitis in non human primates. I: Studies on the relationship of immunoregulation and disease activity. J Immunol 128:116–122, 1982.
10. Wilson B, Huang C, Moore V, Calvanico N: The development of granulomatous pulmonary inflammation in rabbits by aerosol challenge. I. Release of plasminogen activator by alveolar macrophages. Cell Immunol 67:90–100, 1982.
11. Band N, Chang M, Mattler P, Burck P, Van Frank R, Simmerman R, Marks C, Boxer L: Monocytes/macrophage-mediated catabolism of fibrinogen and fibrin. Ann NY Acad Sci 370:568–587, 1981.
12. Unkeless J, Gordon S, Reich E: Secretion of plasminogen activator by stimulated macrophages. J Exp Med 139:834–850, 1974.
13. Myrik Q: Function of the alveolar macrophage in immunity. J Reticulendothel Soc 11:159–175, 1972.
14. Cohn Z, Wiener F: The particulate hydrolases of macrophages. J Exp Med 118:991–1012, 1963.
15. Schuyler M, Steinberg D: Activated alveolar macrophages: IgG and complement receptors. J Lab Clin Med 100:932–942, 1982.
16. Schumacher G, Schill W: Radial diffusion in gel, for micro determination of enzymes. II. Plasminogen activator, elastase and nonspecific protease. Anal Biochem 48:9–46, 1972.
17. Granelli-Piperno A, Reich E: A study of protease and protease–inhibitor complexes in biological fluids. J Exp Med 148:223–234, 1978.
18. Weber K, Osborn M: The reliability of molecular weight determinations by dodecyl sulfate–polyacrylamide gel electrophoresis. J Biol Chem 244:4406–4412, 1969.
19. Goldstein A: Biostatistics. An Introductory Text. New York, Macmillan, 1964.
20. Vassili J, Hamiliton J, Reich E: Macrophage plasminogen activator: Induction by concanavalin A and phorbol myristate acetate. Cell 11:695–705, 1977.

21. Hocking W, Golde D: The pulmonary–alveolar macrophage. N Engl J Med 301:582–587, 1979.

22. Newman W, Gordon S, Hammerling U, Senik A, Bloom B: Production of migration inhibition factor (MIF) and an inducer of plasminogen activator (IPA) by subsets of T cells in MLC. J Immunol 120:927–931, 1978.

23. Vassili J, Reich E: Macrophage plasminogen activator: Induction by products of activated lymphoid cells. J Exp Med 145:429–437, 1977.

24. Bernik B, Wiingaards, Rijken D: Production by human tissue in culture of immunologically distinct multiple molecular weight forms of plasminogen activators. Ann NY Acad Sci 370:592–608, 1981.

25. Hoidal J, Beall G, Rasp F, Holmes B, White J, Repine J: Comparison of the metabolism of alveolar macrophages from humans, rats, and rabbits: Response to heat-killed bacteria or phorbol myristate acetate. J Lab Clin Med 92:787–794, 1978.

26. Miskin R, Reich E: Plasminogen activator: Induction of synthesis by DNA damage. Cell 19:217–224, 1980.

27. Jaken S, Black P: Regulation of plasminogen activator in 3T3 cells: Effect of phorbol myristate acetate on subcellular distribution and molecular weight. J Cell Biol 90:727–726, 1981.

28. Rijken D, Collen D: Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. J Biol Chem 246:7035–7040, 1981.

29. Laug W, Tokes Z, Benedict W, Sorgente N: Anchorage dependent growth and plasminogen activator production by bovine endothelial cells. J Cell Biol 84:281–293, 1980.

30. Allison D, Dirkson J, Schmelter R, Makoid M, Murray W: Production of plasminogen activator from monkey kidney cell culture. Thromb Res 19:525–534, 1980.

31. Virgi M, Vassali J, Estensen R, Reich E: Plasminogen activator of islet of Langerhands: Modulation by glucose and correlation with insulin production. Proc Natl Acad Sci USA 77:875–879, 1980.

32. Lewis J, Pizzo, Adams D: A simple and sensitive assay employing stable reagents for quantification of plasminogen activator. Am J Clin Pathol 76:403–409, 1981.

33. Cohen, S, Isreal E, Spiess-Meir R, Wainberg M: Plasminogen activator is an apparent lymphocyte mitogen. J Immunol 126:1415–1420, 1981.

34. Dvorak H, Orenstein N, Rypyse J, Colvin R, Dvorak M: Plasminogen activator of guinea pig basophilic leukocytes: Probable localization to the plasma membrane. J Immunol 120:766–773, 1978.

35. Neiwoehner D, Cosio M: Chronic obtrusive lung disease: The role of airways disease, with special emphasis on the pathology of small airways. In: Thurlbeck W, Abell M, eds., The Lung, Structure, Function and Disease. Baltimore, Williams & Wilkins, 1978, pp. 160–179.