tem analysis aided our interpretation of the data, the direct in vivo measurements provide new information concerning dopamine compartmentalization. The rapid clearance of dopamine confirms suggestions (*2, 5*) that the detection of extracellular dopamine at micromolar levels is unlikely except under extreme conditions. This work demonstrates that in vivo electrochemistry can be used to monitor the processes of neurotransmitter synthesis, storage, release, and transport, although our methods have yet to be found useful for monitoring the effects of neurotransmitter reuptake. The existence of functional and nonfunctional dopamine compartments available for exocytosis has long been hypothesized (*8–11*). Our data confirm the existence of these compartments and indicate that the nonfunctional compartment is available for stimulated release by the action of amfonelic acid.

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References and Notes

1. R. N. Adams, *Anal. Chem.* **48**, 1126A (1976).
2. A. G. Ewing, R. M. Wightman, M. A. Dayton, *Brain Res.* **249**, 361 (1982).
3. A. G. Ewing, M. A. Dayton, R. M. Wightman, *Anal. Chem.* **53**, 1842 (1981).
4. M. A. Dayton, A. G. Ewing, R. M. Wightman, *J. Electroanal. Chem.* **146**, 189 (1983).
5. F. Gonon, M. Buda, R. Cespuglio, M. Jouvet, J.-F. Pujol, *Nature (London)* **286**, 902 (1980).
6. L. J. Pelligrino and A. J. Cushman, *A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967).
7. R. M. Wightman, E. Strope, P. M. Plotsky, R. N. Adams, *Nature (London)* **262**, 145 (1976); J. C. Conti, E. Strope, R. N. Adams, C. A. Marsden, *Life Sci.* **23**, 2705 (1978); C. A. Marsden, J. Conti, E. Strope, G. Curzon, R. N. Adams, *Brain Res.* **171**, 85 (1979); P. J. Knott, P. H. Hutson, R. P. Scraggs, G. Curzon, in *Function and Regulation of Monoamine Enzymes, Basic and Clinical Aspects*, E. Usdin, N. Weiner, M. B. H. Youdim, Eds. (Macmillan, New York, 1981), pp. 771–780.
8. F. Javoy and J. Glowinski, *J. Neurochem.* **18**, 1305 (1971); P. A. Shore and R. L. Dorris, *Eur. J. Pharmacol.* **30**, 315 (1975); E. S. Sears and P. A. Shore, *J. Pharm. Pharmacol.* **27**, 718 (1975).
9. M. J. Besson, A. Cheramy, P. Feltz, J. Glowinski, *Proc. Natl. Acad. Sci. U.S.A.* **62**, 741 (1969).
10. M. D. Aceto, I. Botton, M. Levitt, R. Martin, H. C. Bentley, P. T. Speight, *Eur. J. Pharmacol.* **10**, 344 (1970); P. A. Shore, *J. Pharm. Pharmacol.* **28**, 855 (1976).
11. H. H. Miller and P. A. Shore, *Eur. J. Pharmacol.* **78**, 33 (1982).
12. J. T. Coyle and S. H. Snyder, *Science* **166**, 899 (1969).
13. J. N. Caviness and R. M. Wightman, *J. Pharmacol. Exp. Ther.* **223**, 90 (1982).
14. J. F. Fischer and A. K. Cho, *ibid.* **208**, 203 (1979).
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A Polypeptide Secreted by Transformed Cells That Modulates Human Plasminogen Activator Production

Abstract. *A diffusible factor produced and secreted by malignant murine cells was capable of inducing plasminogen activator production by normal diploid human fibroblasts. The factor's ability to induce plasminogen activator was insensitive to treatment with nucleases, but its activity was destroyed by digestion with proteases. It is proposed that such a factor would play a role in malignancy if it would recruit normal cells that were adjacent to transformed cells to produce plasminogen activator which could result in tumor-promoted proteolysis.*

Plasminogen activator (PA), a serine protease, causes the conversion of plasminogen to plasmin which in turn is responsible for fibrinolysis. This process is implicated in a number of systems requiring controlled local proteolysis, including ovulation (*1*), embryo implantation (*2*), inflammation (*3*), cell migration and tissue remodeling (*4*), and the conversion of proinsulin to insulin (*5*). The production and secretion of PA is also correlated with certain human disorders such as arthritis (*6, 7*) and the expression of transformed phenotypes (*8*). The enzymatic activity can be readily assayed and the use of an electrophoretic system enables distinction between species-specific forms of PA (*9*). We showed previously that in somatic cell hybrids between PA^+ mouse cells and PA^- human cells, the human form of PA was expressed if human chromosome 6 was present (*10*). Here we show that the modulation of human PA can be mediated by a factor produced and secreted by a variety of malignant cells. The activity of this factor is insensitive to treatment with nucleases but can be destroyed with trypsin.

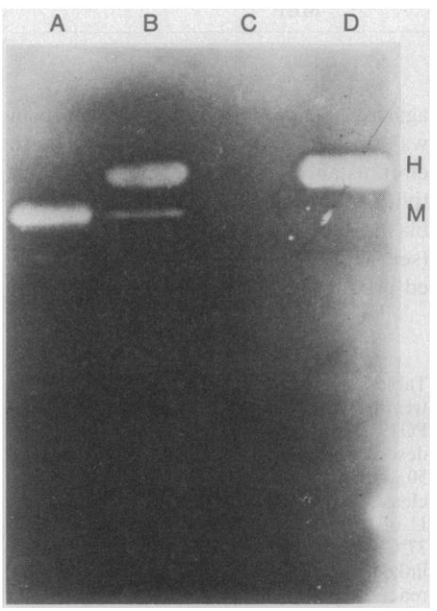

Fig. 1. Induction of PA production in normal human diploid fibroblasts. Cell sources, tissue culture, and the preparation of cell extracts were as described (*10*). Extracts were supplemented with sample buffer and subjected to sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis according to Laemmli (*11*). The gel was washed to remove SDS and was placed on an indicating agar layer containing fibrin, according to the method of Granelli-Piperno and Reich (*9*). After incubation at 37°C, clear bands of lysis were visible in the cloudy fibrin background, corresponding to the position of PA bands in the polyacrylamide gel. The fibrin agar layer was stained with amido black for photography. *H* and *M* mark the position of the predominant human and mouse PA bands, corresponding in size to 60,000 and 48,000 daltons, respectively. (A) PG19 cells (a line derived from a mouse melanoma). (B) Human diploid fibroblasts incubated for 17 hours with cell-free PG19 conditioned medium. (C) Human diploid fibroblasts. (D) HT1080 cells (a line derived from a human fibrosarcoma) positive for PA.

We initially observed modulation of human PA during attempts to map the gene for human PA. We found that production and secretion of human PA can be induced by cell fusion. Cell hybrids between PG19, a melanoma-derived PA^+ mouse cell line, and PA^- normal diploid human fibroblasts produced mouse PA. Several of these hybrids also synthesized human PA. Since the human parental cells were PA^-, we concluded that some cellular factor produced by PG19 is capable of modulating human PA.

We have examined the nature of the activation of the human PA by mouse cells. The PA was assayed by quantitative methods (*3*) and qualitatively by polyacrylamide gel electrophoresis (*11*) followed by a fibrin agar overlay technique (*9*). We wished to determine whether cell fusion is necessary to cause the observed PA modulation, or if the malignant murine cells were secreting a diffusable modulating factor. Actively growing human diploid fibroblasts were incubated in the presence of cell-free, serum-free conditioned culture medium from PG19. The normally PA^- human fibroblasts produced high levels of PA under these conditions (Fig. 1). When the culture media or cell extracts from the two cell types were mixed and then incubated at 37°C, no human PA was

Table 1. Activation of plasminogen activator by different cell lines. Donor cells were grown to 60 percent confluence. The medium (Dulbecco's minimum essential medium with 10 percent fetal calf serum) was removed and was replaced with serum-free medium (SFM), after three rinses with SFM to remove all serum. After 20 hours of incubation, the conditioned culture medium was collected and centrifuged to remove cells and cellular debris. The conditioned culture medium was placed on SFM-washed confluent cultures of the recipient cell lines and incubated for 17 hours at 37°C. PG19 is a mouse melanoma–derived cell line; B82 is a mouse fibrosarcoma–derived cell line; Bowes is derived from a human spontaneous melanoma; and 4-71 is a rat neural tumor–derived cell line. Cell extracts were prepared and assayed as described in Fig. 1. HDF, human diploid fibroblasts; Hamster EF, hamster embryo fibroblasts; MEF, mouse embryo fibroblasts.

Name	Donor cell line (CM)		Recipient cell line	Activation of PA
	Origin	Produces PA		
PG19	Mouse	+	HDF	+
B82	Mouse	−	HDF	+
3T3	Mouse	−	HDF	−
Fibroblasts	Mouse	−	HDF	−
HDF	Human	−	HDF	−
PG19	Mouse	+	Hamster EF	+
Bowes	Human	+	Hamster EF	+
4-71	Rat	+	Hamster EF	+
Fibroblasts	Hamster	−	Hamster EF	−
Bowes	Human	+	MEF	+
4-71	Rat	+	MEF	+
Fibroblasts	Mouse	−	MEF	−

produced. These results indicate that (i) a diffusible factor produced and secreted by mouse PG19 cells is capable of inducing the production of human PA, and (ii) such induction is not due to conversion of a precursor to PA. To ascertain whether the ability to modulate PA is restricted to PG19 cells we tested the effects of serum-free culture fluids from several different transformed and normal cells on human, hamster, or mouse fibroblasts (Table 1). Both intra- and interspecific test systems yielded qualitatively similar results, indicating that the ability to activate and the ability to respond can cross species boundaries.

The diffusible factor is stable since short incubations (up to 24 hours) at 37°C or prolonged storage at −20°C did not eliminate the activating capability of PG19 conditioned medium. To estimate the size of the factor we dialyzed the harvest fluids of PG19 to different exclusion volumes and tested the dialysate and the retentate for PA-inducing activity. On the basis of these results we estimate the size of the diffusible factor to be between 50,000 and 100,000 daltons.

To study the chemical nature of the diffusible factor, we treated the culture fluids with deoxyribonuclease or ribonuclease. Under conditions where these enzymes destroyed their natural substrates (as determined by acid solubility of labeled substrates), the ability of culture fluids to induce PA was unaffected. To test whether the diffusible factor is a polypeptide, we incubated culture fluids with well-washed trypsin-conjugated agarose beads. No detectable trypsin was released from the beads under these circumstances. Both the mouse PA activity and the inducibility of human fibroblasts were lost upon trypsin treatment (see Table 2). As PA is the major secreted product of PG19 cells, it is appropriate to examine the possibility that murine PA may be responsible for the activation of the human PA gene. In our studies with the cell hybrids (*10*), we observed some hybrids that produced mouse PA and contained human chromosome 6 but did not produce human PA. These hybrids could be induced to produce human PA when grown in the presence of serum-free PG19 conditioned medium. The inability of the human PA to be induced by mouse PA indicates that the diffusible factor is distinct from mouse PA. Further, mouse B82 cells, which also produce this factor, are PA^-. These results together indicate that a nondialyzable polypeptide factor secreted by a variety of tumor cells is capable of modulating human PA.

Table 2. A summary of the results obtained by treating the diffusible factor with enzymes. PG19 conditioned medium was prepared as described in Table 1 and then incubated with 50 μg of ribonuclease or 6 μg of deoxyribonuclease per milliliter for 30 minutes, or with 2.5 U of trypsin per milliliter for 1 hour, all at 37°C. The trypsin was immobilized on cross-linked agarose beads (Sigma). The nuclease reactions were halted by addition of EDTA (1 to 10 m*M*) and the trypsin reaction was halted by centrifugation of the suspension. The conditioned media (treated and untreated) were placed on confluent cultures of normal human fibroblasts (HDF) and incubated for 17 hours at 37°C. Cell extracts were prepared and samples were subjected to the qualitative PA assay as described in Fig. 1.

Sample	Mouse PA	Human PA
PG19 (mouse melanoma derived)	+	−
Human diploid fibroblasts	−	−
PG19 conditioned medium (untreated) on HDF	+	+
Deoxyribonuclease-treated PG19 conditioned medium on HDF	+	+
Ribonuclease-treated PG19 conditioned medium on HDF	+	+
Trypsin-treated PG19 conditioned medium on HDF	−	−

Human PA can be detected in the cells 6 to 8 hours after the addition of PG19 conditioned medium. Its production reaches a peak at 12 hours. These results are consistent with regulation of PA at the transcriptional or posttranscriptional level. The diffusible factor acts in a fashion similar to other polypeptide factors in that its action is reversible, that is, human cells stop producing PA if the conditioned medium is replaced by fresh medium.

Several factors are known to induce PA. These include peptide hormones (*1, 12*), adenosine 3′,5′-monophosphate (cyclic AMP) (*1, 13*), irradiation (*14*), carcinogenic promoters (*15*), physical carcinogens (*16*), and other mutagens (*14*). Of these, peptide hormones and cyclic AMP are natural cell products. Because of its large size and sensitivity to trypsin, the diffusible factor is not cyclic AMP. That the factor may be a peptide hormone needs closer examination. At least three different peptide hormones [epidermal growth factor (EGF) (*12*), calcitonin (*17*), and colony-stimulating factor (CSF) (*18*)] are known to induce PA. The diffusible factor cannot be EGF or calcitonin because (i) both of them are dialyzable under the conditions we have used (whereas the factor is not), and (ii) the human fibroblasts we used failed to produce PA upon incubation with purified EGF (5 ng/ml). It is possible that the factor is CSF but there is no direct information that CSF is secreted by a large number of transformed cells. This aspect needs further investigation.

It has recently been shown that protein factors released from stimulated mononuclear phagocytes can induce PA in synovial fibroblasts (*7*). Synovial tissue itself is reported to produce a factor that enhances PA production (*6*). The identity of these factors has not been established. Although PA is implicated

in a variety of biological functions, it mediates these functions through controlled local proteolysis. The fact that different cells can produce PA and that each cell type may respond to different physiological signals may provide the basis for the diverse modes of its activation.

A number of observations implicate production of PA and the concomitant local proteolysis in the onset and maintenance of malignancy (*8*). The discovery that not all transformed cells produce PA cast some doubt on the validity of the proposed role of PA in transformation (*19*). The observations reported here, however, suggest a possible alternative to the necessity of PA production by the malignant cell. The production of a diffusible factor that can induce adjacent normal cells to produce PA would obviate the need for transformed cells to produce PA. Indeed, the production of a diffusible factor may be a more efficient method to achieve tumor-promoted local proteolysis.

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References and Notes

1. W. H. Beers, S. Strickland, E. Reich, *Cell* **6**, 387 (1975).
2. S. Strickland, E. Reich, M. I. Sherman, *ibid.* **9**, 231 (1976).
3. J.-D. Vassalli, J. Hamilton, E. Reich, *ibid.* **8**, 271 (1976).
4. L. Ossowski, J. Quigley, E. Reich, in *Proteases and Biological Control*, D. Rifkin, E. Reich, E. Shaw, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1975), pp. 909–913; J. E. Valinsky, E. Reich, N. M. LeDovarin, *Cell* **25**, 471 (1981).
5. M. A. J. Virgi, J.-D. Vassali, R. D. Estensen, E. Reich, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 875 (1980).
6. J. E. Meats, M. B. McGuire, R. G. G. Russell, *Nature (London)* **286**, 891 (1980).
7. J. A. Hamilton and J. Slywka, *J. Immunol.* **126**, 851 (1981); J. A. Hamilton *et al.*, *J. Exp. Med.* **155**, 1702 (1982).
8. J. C. Unkeless, A. Tobia, L. Ossowski, J. P. Quigley, D. B. Rifkin, E. Reich, *J. Exp. Med.* **137**, 85 (1973); D. B. Rifkin, L. P. Beal, E. Reich, in *Proteases and Biological Control*, D. Rifkin, E. Reich, E. Shaw, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1975), pp. 841–847; R. Pollack, R. Risser, S. Conlon, D. B. Rifkin, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4792 (1974); P. A. Jones, W. E. Laug, W. F. Benedict, *Cell* **6**, 245 (1975); L. Ossowski *et al.*, *J. Exp. Med.* **138**, 1056 (1973).
9. A. Granelli-Piperno and E. Reich, *J. Exp. Med* **147**, 223 (1978).
10. R. Kucherlapati, R. Tepper, A. Granelli-Piperno, E. Reich, *Cell* **15**, 1331 (1978).
11. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
12. L. S. Lee and I. B. Weinstein, *ibid.* **274**, 696 (1978).
13. D. B. Rifkin, *J. Cell. Physiol.* **97**, 421 (1978).
14. R. Miskin and E. Reich, *Cell* **19**, 217 (1980).
15. M. Wigler, D. DeFeo, I. B. Weinstein, *Cancer Res.* **38**, 1434 (1978).
16. J. Hamilton, J.-D. Vassalli, E. Reich, *J. Exp. Med.* **144**, 1689 (1976).
17. J.-M. Dayer, J. D. Vassali, J. L. Bobbitt, R. N. Hull, E. Reich, S. M. Krane, *J. Cell Biol.* **91**, 195 (1981).
18. H.-S. Lin and S. Gordon, *J. Exp. Med.* **150**, 231 (1979); J. A. Hamilton, E. R. Stanley, A. W. Burgess, R. K. Shadduck, *J. Cell. Physiol.* **103**, 435 (1980).
19. D. M. Mott, T. H. Fabisch, B. P. Sani, S. Sorof, *Biochem. Biophys. Res. Commun.* **61**, 621 (1974).
20. We thank A. Granelli-Piperno and E. Reich for assistance with some early assays of PA and G. Grosse for technical assistance. Work supported by NIH grants CA-35171 (R.K.) and CA-16239 (D.B.R.) and grants from the American Cancer Society, National Science Foundation, and March of Dimes Birth Defects Foundation. R.L.D. was the recipient of an NSF predoctoral fellowship.
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Herpes Simplex Virus Glycoprotein D: Human Monoclonal Antibody Produced by Bone Marrow Cell Line

Abstract. *Normal bone marrow cells from a donor positive for herpes simplex virus were transformed with Epstein-Barr virus. The resulting lymphoblastoid cell line has secreted immunoglobulin G_1 of the κ type continuously for 2 years. This immunoglobulin, detected both on the cell surface and in the cytoplasm, reacts with cells infected with herpes simplex virus. It defines an antigen that comigrates with the 55-kilodalton glycoprotein D of herpes simplex virus type 1 and neutralizes the infectivity of herpes simplex viruses 1 and 2.*

Normal human B lymphocytes, usually from peripheral blood, can grow continuously in vitro after infection with Epstein-Barr virus (EBV) (*1*). After such immortalization, these lymphoblastoid cell lines are able to synthesize and secrete immunoglobulins (Ig's) (*2, 3*), some of which show a specific antibody activity (*4–6*). Synthesis of Ig in vivo occurs in spleen and lymph nodes early in life and later mainly in bone marrow. Since human bone marrow is considered to be the major source of the different Ig classes in the serum (*7*), lymphoid cells from this source were isolated, infected in vitro with EBV, and established as permanent lines. We report here that a lymphoblastoid cell line obtained from normal bone marrow lymphocytes secretes a monoclonal IgG_1 κ at an unusually high rate, and without any cloning. This monoclonal antibody is directed against the glycoprotein D of herpes simplex virus (HSV). Since the glycoprotein D is common to both HSV-1 and HSV-2, the antibody neutralizes the infectivity of both types and thus might represent a means to protect subjects at the moment of exposure to HSV-1 and HSV-2.

The bone marrow was obtained by aspiration from the sternum from a 73-year-old patient with anemia who had been treated 7 years previously for renal carcinoma. The aspirate was passed through glass filters and washed three times, and the granules were dispersed by repeated aspiration through progressively narrower bore needles. Total counts of nucleated cells and differential counts confirmed the normal composition of the bone marrow; more than 90 percent were living cells. For transfor-

Table 1. Analysis of Ig synthesis in Ri-BM cell line. Surface-bound Ig's were detected with direct membrane immunofluorescence (IF) on fresh cells by using monospecific fluorescein-conjugated rabbit $F(ab')_2$ fragments of antibodies to human γ, μ, α, δ, κ, and λ chains. Intracytoplasmic Ig's were detected by indirect IF on acetone-fixed cells with the same antisera. The specificity of the antisera was tested on well characterized myeloma or chronic lymphocytic leukemia cells and used at different dilutions accordingly. N.D., not determined.

Ig	Surface Ig (% of positive cells)	Intracytoplasmic Ig (% of positive cells)	Ig in supernatant in 24 hours (μg/10^6 cells)*
D	< 0.1	< 0.1	< 1
M	< 0.1	< 0.1	< 1
G	92	89	15 (IgG_1 κ only)†
A	< 0.1	< 0.1	< 1
κ	100	94	N.D.
λ	< 0.1	< 0.1	N.D.

*After the correct concentration was achieved with an Amicon DC 2 concentrator (hollow fiber type H1 P10-8) and then an YP_{10} membrane concentrator under positive nitrogen pressure, the supernatants were tested for the presence of Ig's by electrophoresis on cellulose polyacetate (Veronal buffer, *p*H 9.8). The IgG, IgA, IgM, and IgD were measured by radial immunodiffusion. The results are related to the nonconcentrated supernatant. †Determination of the 1, 2, 3, and 4 subclasses of IgG was achieved by Ouchterlony analysis in 1.5 percent agar gel with the specific antisera (Organon).