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.

ROBIN L. DAVIES
Department of Biochemical Sciences, Princeton University, Princeton, New Jersey 08544, and *Center for Genetics, University of Illinois College of Medicine, Chicago 60612*
DANIEL B. RIFKIN
Department of Cell Biology, New York University School of Medicine, New York 10016
ROBERT TEPPER*
ANNE MILLER*
RAJU KUCHERLAPATI
Department of Biochemical Sciences, Princeton University, and *Center for Genetics, University of Illinois College of Medicine*

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.
* Present address: Harvard Medical School, Boston, Mass. 02115.

17 February 1983; revised 11 April 1983

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).

Table 2. Specificity of the antibody to HSV in the patient's serum and in the Ri-BM supernatant. DEAE-cellulose ion exchange chromatography was used to isolate the IgG's from the culture supernatant in 0.2*M* tris-HCl buffer, *p*H 8.6, containing 0.015*M* NaCl. The IgG fraction and the fraction without IgG were dialyzed against phosphate-buffered saline, *p*H 7.4, and reconcentrated (as described in Table 1). Herpes simplex virus type 1 (HSV-1) antibodies were detected by the following techniques: indirect IF on Raji (A44) cell line (*19*) and on virus-infected MRC-5 cells, ELISA test, and neutralization of cytopathic effect. Results are expressed as reciprocals of the antibody titer.

Condition	Indirect IF* (HSV-1)				ELISA (HSV-1)		Neutralization	
	μ	γ	κ	λ	μ	γ	HSV-1	HSV-2
Supernatant (IgG_1 κ) (nonconcentrated)	< 1	64	64	< 1	< 1	256	8	4
Serum	< 8	256	256	32	< 16	> 512	64	16

*$F(ab')_2$ fragments for fluorescein conjugates (see Table 1).

mation in vitro, the supernatant of a culture of B 95-8 cells was used, as previously described (*1*). A lymphoblastoid cell line was established within 10 days from bone marrow cells infected with EBV and was called Ri-BM. Although the patient's serum was EBV-positive (antibody to viral capsid antigen 640, antibody to Epstein-Barr nuclear antigen 160), no cell line from the bone marrow was obtained spontaneously, that is, without addition of EBV. The characteristics of the cells of the Ri-BM line were studied at 5 and 18 months after continuous culture and were found to be similar. Histochemically, 35 percent of the cells stained with periodic acid–Schiff reagent, all the cells stained for acid phosphatase and none stained for myeloperoxidase. No chromosomal structure alteration was observed (karyotype 46, XY). One hundred percent of the Ri-BM cells synthesized Epstein-Barr nuclear antigen (*8*), 7 percent early antigens, and < 0.1 percent viral capsid antigen (*9*). Most of them expressed C_3b receptors (90 percent were EAC rosette-forming cells, that is, with erythrocytes, antibody, and complement) (*10*). In an ultrastructural study, some of the cell sections showed swollen cisternae with many associated ribosomes that had invaded the cytoplasmic matrix. Immunoglobulins were detected both on the surface of cells and inside the cytoplasm; Ri-BM cells expressed only γ and κ chains. Immunoglobulin G was detected in the culture medium; this IgG belonged to the IgG_1 subclass and was of the κ type (Table 1).

The patient's serum contained antibodies against HSV, cytomegalovirus, varicella-zoster virus, EBV, papovavirus BK, rubella and measles viruses, and polioviruses. We therefore tested the antibody activity of the subclass-restricted IgG_1 κ produced in the culture supernatant against all these antigens. This IgG_1 κ was positive only with HSV; enzyme-linked immunosorbent assay (ELISA) showed that only IgG antibodies were bound to HSV antigens, and indirect immunofluorescence revealed only IgG κ antibodies, although the patient's serum had a normal content of IgG κ and IgG λ antibodies to HSV (Table 2). After fractionation of the supernatant on a DEAE-cellulose column, nearly all the antibody activity was detected in the IgG fraction. The IgG_1 κ showed neutralizing activity against HSV-1 and HSV-2 in the absence of active complement (Table 2).

To better define the nature of the antigenic determinant recognized by the antibody secreted by the Ri-BM cells, we used the antibody to precipitate either HSV-1– or HSV-2–infected cells radioactively labeled with [^{35}S]methionine or [^{14}C]glucosamine. The electrophoretic pattern of [^{35}S]methionine-labeled

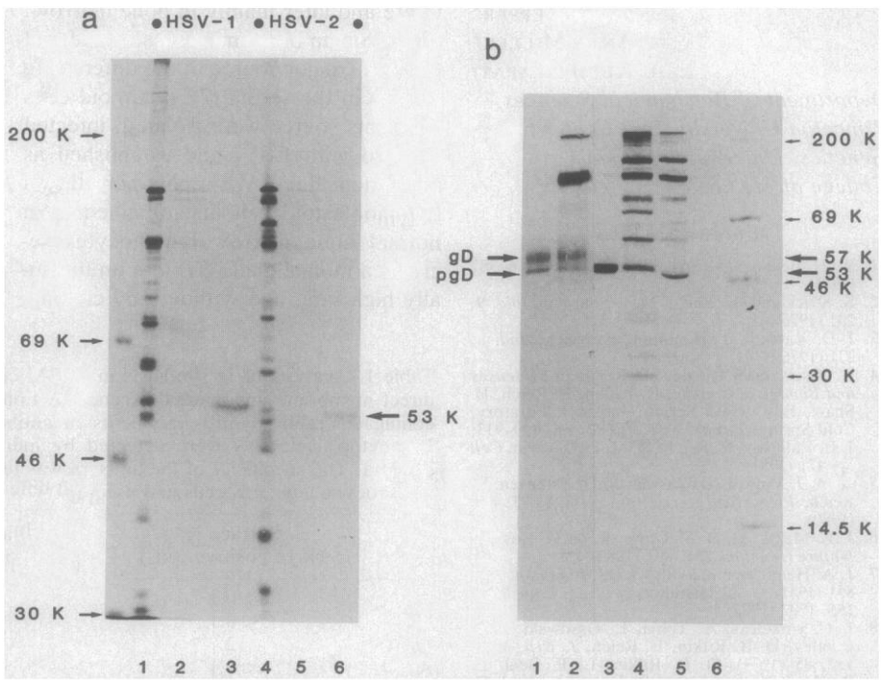

Fig. 1. Autoradiograms for characterization of the monoclonal antibody. Hep-2 cells were infected with HSV-1 (F strain) or HSV-2 (G strain) at 20 plaque-forming units per cell. After incubation for 1 hour at 37°C, virus suspension was removed and the cells were overlayed with maintenance medium. At 16 hours after infection, the cells were radioactively labeled for 1 hour with [^{35}S]methionine (50 μCi/ml; NEN 1000 mCi/mmole) in Medium-199 containing 10 percent methionine supplemented with 1 percent dialyzed fetal calf serum (FCS). The cells were also labeled, from 3 to 24 hours after infection, with [^{14}C]glucosamine (1 μCi/ml; NEN 60 mCi/mmole) in Medium-199 supplemented with 1 percent dialyzed FCS. At the end of the labeling period the cells were washed three times with phosphate-buffered saline and then mixed for 1 hour at 4°C in extraction buffer, 0.1*M* tris-(hydroxymethyl)aminomethane-hydroxychloride (*p*H 8.0), 10 percent (by volume) glycerol, 0.5 percent Nonidet-P40, 0.5 percent sodium desoxycholate, and 0.2 m*M* phenylmethylsulfonyl fluoride. After centrifugation at 60,000*g* for 1 hour, the extracts were incubated with the supernatant of cells producing monoclonal antibody or with the serum of the patient and subsequently treated with Protein A of *Staphylococcus aureus* (10 percent solution). The immunoprecipitated proteins were separated by electrophoresis on an 8.5 percent polyacrylamide slab gel containing sodium dodecyl sulfate and detected by fluorography with Kodak SB 5 x-ray film (*20*). (a) Immunoprecipitated and total viral proteins (infected cells labeled with [^{35}S]methionine). Cells infected with HSV-1 (lanes 1, 2, and 3) or HSV-2 (lanes 4, 5, and 6), without immunoprecipitation (lanes 1 and 4), after immunoprecipitation by patient's serum (lanes 2 and 5), and by monoclonal antibody (lanes 3 and 6). (b) Immunoprecipitated and total viral proteins (HSV-1–infected cells labeled with [^{14}C]glucosamine) (lanes 1 and 2) [^{35}S]methionine labeled HSV-1–infected cells (lanes 3, 4, 5, and 6), without immunoprecipitation (lanes 1 and 3), after immunoprecipitation by patient's serum (lane 5), and after treatment with a negative serum (lane 6).

proteins precipitated with IgG_1 κ revealed a protein that comigrates with a known structural protein of HSV-1 (55,000 daltons) (Fig. 1a).

With [^{14}C]glucosamine labeling, the monoclonal antibody was found to react with two glycoprotein bands, designated gD and pgD, the latter being the precursor of gD (Fig. 1b). Immunoprecipitation with HSV-2–infected cells showed that the specificity encompasses the two serological types of HSV (Fig. 1a), as already described by Eisenberg *et al.* for several mouse monoclonal antibodies against HSV (*11*). The antibody production has remained stable for a 2-year period, and the antibody specificity is preserved after freezing.

These results indicate that it is possible to obtain EBV-transformed bone marrow cells that continuously secrete a high and stable amount (15 μg per 10^6 cells per 24 hours) of human antibodies. Since few human myeloma lines are available for producing Ig-secreting human-human hybridomas, the transformation of human immune B lymphocytes by EBV appears to be a good approach to the production of human monoclonal antibodies. In general, lymphoblastoid cell lines from peripheral blood secrete polyclonal antibodies (*12*), but cloning should allow monoclonality. Since the correlation between the distribution profile of the cytoplasmic Ig cells and the levels of various Ig's in the serum is better in the bone marrow than in the other peripheral lymphoid tissues, the bone marrow, whenever it is available, seems to be a good source (*7, 13–15*) for establishing antibody-secreting lymphoblastoid cell lines. Such a line could not be obtained from peripheral blood lymphocytes. The small number of T lymphocytes in bone marrow may facilitate establishing cell lines. As has been observed recently (*16*), bone marrow contains more committed B cells than blood which contains more virgin B cells.

It is possible that oligoclonal cell selection in the bone marrow had occurred in vivo as a result of the immunodepressed status of the 73-year-old cancer patient. Alternatively, a premyelomatous state might have begun at the time we obtained the bone marrow. We cloned this Ri-BM cell line in vitro; most of the clones were secreting but not at a high level. The HSV antigenic stimulus probably occurred outside the bone marrow, since there is no local induction of antibody formation in the bone marrow but an immigration of antigen-activated B cells. From the 32 human lymphoblastoid cell lines that we have now established from HSV-positive donors, we have just obtained a second cell line producing a monoclonal antibody to HSV gD. Recently, the gene of this protein was mapped and a gD-related polypeptide was synthesized (*17*). A murine gD monoclonal antibody protects mice against HSV-induced neurological diseases (*18*) and thus a human gD monoclonal antibody that protects against both HSV-1 and HSV-2 might provide a means of passive immunization against HSV infections in humans.

J. M. SEIGNEURIN
Laboratoire de Virologie, Centre Hospitalier Universitaire de Grenoble, 38043 Grenoble, France
C. DESGRANGES
Laboratoire EBV, Faculté de Médecine Alexis Carrel, Lyon, France
D. SEIGNEURIN
Laboratoire d' Hématologie, Centre Hospitalier Universitaire de Grenoble
J. PAIRE
Laboratoire EBV, Faculté de Médecine Alexis Carrel
J. C. RENVERSEZ
Laboratoire de Biochimie, Centre Hospitalier Universitaire de Grenoble
B. JACQUEMONT
Laboratoire EBV, Faculté de Médecine Alexis Carrel
C. MICOUIN
Laboratoire d' Immunologie, Centre Hospitalier Universitaire de Grenoble

References and Notes

1. G. Miller and M. Lipman, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 190 (1973).
2. A. Rosen, P. Gergely, M. Jondal, G. Klein, S. Britton, *Nature (London)* **267**, 52 (1977).
3. N. A. Brown and G. Miller, *J. Immunol.* **128**, 24 (1982).
4. V. R. Zurawski, Jr., E. Haber, P. H. Black, *Science* **199**, 1439 (1978).
5. M. Steinitz, S. Koskimies, G. Klein, O. Mäkelä, *J. Clin. Lab. Immunol.* **2**, 1 (1979).
6. M. Steinitz, G. Izak, S. Cohen, M. Ehrenfeld, I. Flechner, *Nature (London)* **287**, 443 (1980).
7. R. Benner, W. Hijmans, J. J. Haaijman, *Clin. Exp. Immunol.* **46**, 1 (1981).
8. B. M. Reedman and G. Klein, *Int. J. Cancer* **11**, 499 (1973).
9. W. Henle and G. Henle, *J. Bacteriol.* **91**, 1248 (1966).
10. M. Jondal and G. Klein, *J. Exp. Med.* **138**, 1365 (1973).
11. R. J. Eisenberg, D. Long, L. Pereira, B. Hampar, M. Sweig, G. H. Cohen, *J. Virol.* **41**, 178 (1982).
12. J. M. Seigneurin, C. Desgranges, J. C. Renversez, M. Baccard, C. Micouin, *Ann. Virol. (Inst. Pasteur)* **132 E**, 323 (1981).
13. R. McMillan, R. L. Longmire, R. Yelenosky, J. E. Lang, V. Heath, C. G. Craddock, *J. Immunol.* **109**, 1386 (1972).
14. A. Morell, F. Skvaril, W. Hijmans, R. Scherz, *ibid.* **115**, 579 (1975).
15. G. C. De Gast and T. A. E. Platts-Mills, *ibid.* **122**, 285 (1979).
16. S. Fong, J. H. Vaughan, C. D. Tsoukas, D. A. Carson, *ibid.* **129**, 1941 (1982).
17. R. J. Watson, J. H. Weis, J. S. Salstrom, L. W. Enquist, *Science* **218**, 381 (1982).
18. R. D. Dix, L. Pereira, J. R. Baringer, *Infect. Immun.* **34**, 192 (1981).
19. J. M. Seigneurin, C. Desgranges, M. F. Lavoué, G. De Thé, *J. Immunol.* **117**, 950 (1976).
20. M. Sweig, C. J. Heilman, J. Rabin, B. Hampar, *J. Virol.* **35**, 644 (1980).
21. We thank M. Baccard and O. Genoulaz for technical assistance, and J. P. Lamelin, G. De Thé, and J. P. Revillard for critical comments. This work was supported by grants from INSERM (126 007) and from Université Scientifique et Médicale de Grenoble.

29 March 1983; revised 25 April 1983

Interaction of Human Hemoglobin and Its Variants with Agar

Abstract. *In citrate agar electrophoresis hemoglobin appears to bind reversibly to the sulfated polysaccharide agaropectin, a natural component of Difco Bacto-Agar. This complex migrates anodally, since the hemoglobin is only weakly positively charged at pH 6.2 whereas the carbohydrate carries a net negative charge. Electroendosmosis, on the other hand, proceeds in the cathodal direction. These opposing fluxes separate the hemoglobins in the order of their affinity for agaropectin. An agaropectin binding site was identified on hemoglobin by computer-assisted modeling, and the relation of the site to hemoglobin variants that exhibit abnormal citrate agar mobility was established. The citrate anion is postulated to function as a "counter ion." Preliminary evidence indicates that agaropectin has antigelling properties with respect to hemoglobin S.*

Citrate agar electrophoresis is widely used for confirming the occurrence of hemoglobin (Hb) S (β6 Glu → Val) and Hb C (β6 Glu → Lys) in the presence of other Hb's and for differentiating these Hb's from variants with similar migration properties on other electrophoretic media (*1*). The method is based on the observation of Robinson *et al.* (*2*) that Hb migration on agar gels at *p*H 6.2 in the presence of citrate anions appears to depend on some factor other than, or in addition to, electrostatic charge. The order of Hb separation, cathode to anode, is F, A (and most variants of A), S, and C (Fig. 1a). Gratzer and Beaven (*3*), who studied the effect of a number of variables on the separation, concluded that electroendosmosis, the electric current–induced movement of buffer in a gel containing an immobilized charge, was probably the driving force and noted that the order of separation resembled the affinity of the Hb's for anionic ion-exchange columns (*4*). In addition, they suggested that the amino terminal of the β chain was involved. Schneider and coworkers (*5, 6*) made some generalizations about the relation between the structure of the Hb variants and their