9. Centers for Disease Control, *ibid.* **36**, 3 (1987).
10. M. E. Chamberland, *J. Am. Med. Assoc.* **254**, 383 (1985).
11. A. M. Hardy, *Public Health Rep.* **102**, 386 (1987).
12. *International Classification of Diseases* (World Health Organization, Geneva, Switzerland, ed. 9, 1977).
13. New York City Department of Health, *Am. J. Epidemiol.* **123**, 1025 (1986).
14. M. Marmor, D. C. Des Jarlais, and S. R. Friedman, Sr. [*AIDS* **11**, 39 (1987)] describe how the cohort was recruited.
15. R. S. Klein, *N. Engl. J. Med.* **311**, 354 (1984).
16. R. A. Kaslow, *Ann. Int. Med.* **107**, 474 (1987).
17. R. R. Redfield, D. C. Wright, E. C. Tramont, *N. Engl. J. Med.* **314**, 131 (1986).
18. New York State Division of Substance Abuse Services, *Statewide Comprehensive Five-year Plan* (Albany, 1987).
19. B. Polsky, *Ann. Int. Med.* **104**, 38 (1986).
20. M. S. Simberkoff, W. El Sadr, G. Schiffman, J. J. Rahal, Jr., *Am. Rev. Resp. Dis.* **130**, 1174 (1984).
21. P. Selwyn *et al.*, "AIDS and HIV-related mortality in intravenous drug users," paper presented at the fourth international conference on acquired immunodeficiency syndrome (AIDS), Stockholm, Sweden, 12 to 16 June 1988.
22. Centers for Disease Control, *Morb. Mortal. Wkly. Rep.* **36**, 785 (1987).
23. M. Seligmann, *Ann. Int. Med.* **107**, 234 (1987).
24. T. A. Peterman, P. Drotman, J. W. Curran, *Am. J. Epidemiol.* **7**, 1 (1985).
25. I. S. Cohen, *N. Engl. J. Med.* **315**, 628 (1986).
26. T. K. S. Rao, E. A. Friedman, A. D. Nicastri, *ibid.* **316**, 1062 (1987).
27. A. Kristal, *J. Am. Med. Assoc.* **255**, 2306 (1986).
28. W. Szmuness *et al.*, *Ann. Int. Med.* **83**, 489 (1975).
29. W. Szmuness *et al.*, *N. Engl. J. Med.* **303**, 833 (1980).
30. C. E. Stevens, unpublished data.
31. New York City Department of Health, *Bureau of Tuberculosis 1986 Annual Report* (New York, 1988).
32. D. C. Des Jarlais, "Alcohol, HIV infection and pneumonia among intravenous drug users," paper presented at the Annual Conference of the American Psychiatric Association, Chicago, May 1987.
33. A full description of this classification is available from R.L.S.

10 June 1988; accepted 18 September 1988

Infection and Replication of HIV-1 in Purified Progenitor Cells of Normal Human Bone Marrow

THOMAS M. FOLKS,* STEVEN W. KESSLER, JAN M. ORENSTEIN, JESSE S. JUSTEMENT, ELAINE S. JAFFE, ANTHONY S. FAUCI

Myeloid progenitor cells were highly purified from normal human bone marrow by positive immunoselection with high-affinity monoclonal antibodies linked to magnetic beads and were successfully infected in vitro with the human immunodeficiency virus type 1 (HIV-1). From 99 to 100 percent pure bone marrow cells expressing the CD34 phenotypic marker were obtained. These cells were devoid of mature myeloid or T cell surface and intracellular markers as analyzed by immunohistochemical staining and flow cytometry. HIV-1 particles were detected by supernatant reverse transcriptase activity and transmission electron microscopy 40 to 60 days after infection. Viral particles were predominantly observed assembling and accumulating from within intracellular membranes, while phenotypically the cells were observed to have differentiated into CD4$^+$ monocytes. These studies have important implications in understanding the pathogenesis of HIV-1 as well as the possible cause of certain of the observed hematologic abnormalities in HIV-1 infection. They also indicate that the bone marrow may serve as a potentially important reservoir of HIV-1 in the body.

A SPECTRUM OF HEMATOLOGIC ABnormalities have been described in patients with the acquired immunodeficiency syndrome (AIDS) and human immunodeficiency virus type 1 (HIV-1)–related disorders. These have included peripheral blood cytopenias of all cell types (*1*) as well as bone marrow abnormalities such as myelodysplasia (*2–4*). Indirect evidence of infection of bone marrow cells has been reported and proposed as a potential explanation for these abnormalities. Busch *et al.* (*5*) detected the presence of HIV-1 mRNA in myelocytes and monomyelocytes from bone marrow of patients with AIDS using in situ hybridization, suggesting that myeloid precursors might be infected. Donahue *et al.* (*6*) reported that immune mediated mechanisms might be responsible for the ineffective hematopoiesis seen in patients with AIDS and AIDS-related complex (ARC). They found that antibodies to the envelope glycoprotein (gp120) of HIV-1 could suppress granulocyte-macrophage colony stimulating factor–dependent colony formation by low density, nonadherent bone marrow cells from HIV-1–infected individuals but not from normal controls. They postulated that antibody to gp120 recognized this virus-encoded protein expressed on the surface of HIV-1–infected progenitor cells, but not on normal cells, and directly or indirectly blocked their growth. In the absence of antibody to gp120, the proliferative potential of the cells was not grossly altered, indicating that HIV-1 may not be directly cytotoxic to the myeloid precursors. This raises the possibility that the bone marrow may serve as a reservoir for HIV-1 in the body.

The ability to study directly the hypothesis that HIV-1 can infect myeloid progenitor cells has been hampered by the difficulty of obtaining these cells in highly purified suspensions devoid of contaminating cells known to be susceptible targets of HIV-1, such as CD4 lymphocytes (*7*) and mature monocytes (*8*). In the present study we examined virtually pure preparations of normal human bone marrow cells expressing the CD34 antigen, which were obtained with a novel positive immunoselection procedure. This small subset of bone marrow cells (0.5 to 2%) is highly enriched in hematopoietic progenitors of all lineages (*9*,

Table 1. Phenotypic profile of fractionated bone marrow cells. Red cells were lysed in buffered ammonium chloride solution. A fraction of Fc receptor bearing cells was magnetically depleted by adherence to preformed human and mouse immune complexes coupled to BioMag beads (Advanced Magnetics). Positive immunoselection was performed with K6.1 (IgG2a) antibody crosslinked onto Dynabeads M-450 (Dynal), and cells were recovered by cleaving the antibody-bead linkage. Antibodies were purchased from Becton Dickinson or Coulter. These were either directly conjugated with fluorescein or phycoerythrin, or if they were of the IgG1 or IgM isotypes were detected with conjugated isotype-specific secondary antibodies (Fisher Biotech). Negative controls were matched for isotype, mode of conjugation, and source. Cells were analyzed on an Ortho Cytofluorograf. Cells that showed less than 1% positivity were considered not detectable (ND). Some additional phenotypic properties of the CD34$^+$ cells have been reported (*11*, *20*).

Monoclonal antibody	Percent positive
Myeloid	
CD34 (MY10)	99
CD13 (MY7)	75
CD33 (MY4)	50
CD11b (M01, CR3)	ND
CD14 (M02, Leu-M3)	ND
T lymphocyte	
CD3 (T3, Leu-4)	ND
CD4 (T4, Leu-3)	ND
CD5 (Leu-1)	ND
CD8 (T8, Leu-2)	ND
Null lymphocyte	
CD16 (Leu-11a)	ND
Class II MHC	
Dr	85–90

T. M. Folks, J. S. Justement, A. S. Fauci, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.
S. W. Kessler, Immunobiology and Transplantation Department, Naval Medical Research Institute, Bethesda, MD 20814.
J. M. Orenstein, Department of Pathology, George Washington University School of Medicine, Washington, DC 20037.
Elaine S. Jaffe, Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

*Present address and to whom correspondence should be addressed: Centers for Disease Control, Building 7, Room 240, 1600 Clifton Road, Atlanta, GA 30333.

10) and does not include any detectable phenotypically or functionally mature cells (*9, 11*) (Tables 1 and 2). We show here that these cells can be directly infected with HIV-1 in vitro and can efficiently propagate the virus in the presence of relatively little cytopathicity.

To obtain a sufficient number of purified cells for concurrent infectivity and phenotypic studies, we collected human bone marrow from vertebral bodies of cadaveric organ donors. After red cell lysis and adherence depletion steps, $CD34^+$ cells in the remaining cell suspension were isolated by adsorption to 4-μm magnetic beads linked via the high-affinity CD34 monoclonal antibody (MAb) K6.1 (*12*) and then by magnetic selection (*11*). Beads were dissociated from the cells chemically under conditions that did not alter the detection of other surface antigens. When reexamined for CD34 expression by immunofluorescence staining with the MAb-MY-10 (*9*), which recognizes a separate epitope on this antigen (*12*), virtually 100% of the cells were positive. The majority of cells were also positive for class II major histocompatibility complex (HLA-DR) as well as myeloid markers CD13 (MY-7) and CD33 (MY-9). However, no cells were detected bearing other myeloid, T lymphocyte, or natural killer cell markers (Table 1). In other experiments where immunohistochemical analysis was performed and fewer cells were required, $CD34^+$ cells were purified from bone marrow aspirates after Ficoll-Hypaque separation. In this procedure red blood cells were removed by Ficoll-Hypaque and the remaining $CD34^+$ cells were further isolated by the magnetic bead procedure described above. Both $CD34^+$ and $CD34^-$ cultured cells were harvested after 24 hours and fixed for immunohistochemical staining. All $CD34^+$ cells were found to be positive for MY-10 and negative for all mature T cell markers tested including CD4. In addition, none of the cells showed α-naphthyl butyrate esterase staining indicative of monocytes. The CD34-depleted population showed some positivity (5 to 10%) for all mature markers tested (Table 2).

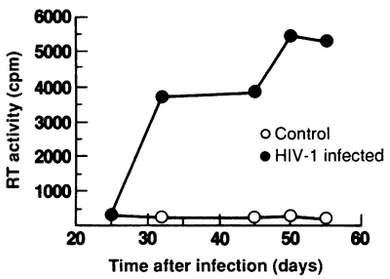

Fig. 1. Kinetics of HIV-1 infection of normal human $CD34^+$ bone marrow cells. The cells were prepared as described (see Table 1) and seeded in Costar 24-well plates at a concentration of 5×10^5 cells per milliliter in 1 ml of medium (Iscove's modified Dulbecco's medium containing 2 m*M* glutamine, 25 m*M* Hepes, and 10% fetal calf serum). Cultures can be maintained for more than 10 weeks under these conditions (*20*). Cell cultures were incubated with a 10^{-1} dilution of virus (100 μl of stock HIV-1) (MOI of approximately 1 IU per ten cells) for 24 hours at 37°C and 7% CO_2; 75% of the medium was removed every 24 hours for the first 5 days and then once every week. Culture supernatants were stored at −70°C. Reverse transcriptase activity was measured weekly by a modification of the technique described by Wiley *et al.* (*21*).

Using a high multiplicity of infection (MOI) (1 IU per ten cells), we incubated stock HIV-1 with the $CD34^+$ cells for 24 hours and cultures were maintained without additional defined growth factors or accessory cells. At approximately 1 month after initiation of HIV-1 infection, reverse transcriptase (RT) activity could be detected in the supernatants of cultures exposed to HIV-1, but not in uninfected control cultures (Fig. 1). A progressive increase in supernatant RT was observed over the remaining period of culture. At no point during the entire culture period was there any evidence of gross cytopathic effects as determined by light microscopy. Between 40 and 60 days of culture, cells from infected and uninfected cultures were harvested and fixed for immunohistochemical analysis. The cells had now converted to a $CD34^-$ and nearly totally (98%) α-naphthyl butyrate esterase–positive phenotype. In addition, this differentiation was associated with conversion of virtually all cells to CD4 positivity. This differentiation occurred in cells from both the infected and uninfected cultures. Staining for CD4 was significantly weaker in the infected culture. At no point in either infected or uninfected cultures were mature T cells (leu 1^+) ever observed (Table 2).

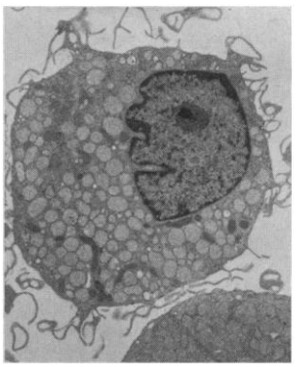

Fig. 2. Transmission electron micrograph of a typical uninfected bone marrow cell at day 75 of culture. There are granular vacuoles filling the cytoplasm, an irregular eccentric nucleus, complicated surface folds, and few lysosomes (×4700).

At 75 days after infection, cultures were harvested and the cells processed for transmission electron microscopy (TEM) (*13*). Uninfected cells, both adherent (generally flattened) and nonadherent, had irregular, complicated surfaces and showed active pinocytosis, eccentric flattened nuclei, small perinuclear Golgi zones, scattered mitochondria and profiles of rough endoplasmic reticulum, relatively few lysosomes, and varying numbers of cytoplasmic vacuoles filled with granular or flocculent material (granular vacuoles) of increasing density (Fig. 2). The Golgi apparatus and pinocytotic vesicles appeared to be involved in the formation of the vacuoles, which often appeared to be enlarging by fusion.

Greater than 50% of cells in cultures exposed to HIV-1 contained typical budding and mature particles with conical-shaped nucleoids (Fig. 3A). Clearly, the exposed plasma membrane in direct contact with the medium was the least common site for virus production, as indicated by the

Table 2. Immuno- and histochemical analysis of HIV-1–infected bone marrow progenitor cells. Positively selected CD34 bone marrow cells were isolated as described (see Table 1). After culture with or without HIV-1 for 40 to 60 days (see Fig. 1) cells were removed from culture wells, centrifuged, and stained with either indirect peroxidase conjugated MAbs (MY10, Leu1, Leu3, Becton Dickinson) or nonspecific esterase for in situ analysis. In addition, freshly selected $CD34^+$ and $CD34^-$ cells were cultured for 24 hours and subjected to similar histochemical analysis. Grading of histochemical analysis was recorded as the percentage of cells showing positive staining (−, 0.5%; +, 5 to 10%; +++, 80 to 90%; ++++, 90 to 99%; ±, 5 to 10%, weak staining). Culture supernatants derived from HIV-1–infected cells were all positive for RT activity. These data represent the successful infection of eight separate bone marrow–derived $CD34^+$ cultures using either LAV, bone marrow–adapted LAV, or a macrophage derived HIV-1 isolate.

Marker	24 hours in culture		40 to 60 days in culture	
	$CD34^+$	$CD34^-$	Uninfected $CD34^+$ cells	Infected $CD34^+$ cells*
CD34 (MY10)	++++	+	−	−
CD5 (Leu 1)	−	+	−	−
CD4 (Leu 3)	−	+	++++	±
Nonspecific esterase	−	+	++++	+++

*Reverse transcriptase–positive.

relatively few budding particles. When present, budding virions could be seen on the lower surface, against the plastic substrate, as well as on the upper surface. By far, the two most active sites of virus production and accumulation were complex invaginations of the cell surface (Fig. 3B) and irregular cytoplasmic spaces that resembled endosomes but did not have associated lysosomes (Figs. 3, C and D). The distinction between the two types of compartments was difficult. The virus-laden compartments appeared capable of progressively increasing in number and size until budding and mature virions virtually replaced the entire cytoplasm (Fig. 3E). Such cells appeared to eventually lyse and detach from the substrate. Virions were less commonly seen in granular vacuoles (Fig. 3F), multivesicular bodies, and smooth vacuoles resembling Golgi elements. Infected cells tended to have fewer granular vacuoles, more primary lysosomes and were more often binucleate and larger in size than their uninfected counterpart.

The cells in the uninfected controls were morphologically homogeneous. Although they had features of myelocytes and especially monocytes, they lacked lysosomes, characteristic cytoplasmic granules, or a prominent Golgi apparatus. Their cytoplasm was dominated by granular vacuoles that appeared to have a relationship to both the Golgi and pinocytic vesicles. The vacuoles resembled the primary granules (PI) described in promyelocytes, but characteristic denser primary and secondary granules were not seen. In contrast, the infected cells tended to have fewer granular vacuoles and more primary lysosomes, suggesting cell maturation as virus production increased.

The virus appeared to assemble preferentially on internal membranes; these were either invaginations of the plasma membrane or sites that appeared to be isolated from the plasma membrane, representing either sealed-off invaginations or phagosomes or autophagosomes. In either case, these compartments were present long enough to accumulate large numbers of virions. It is likely that a small, and perhaps steady, proportion of infected cells reached a degree of intracompartmental virus production and accumulation that was not compatible with survival and released large numbers of virions into the medium. In this regard, culture supernatants from infected cultures were transferred onto susceptible phytohemagglutinin-stimulated peripheral blood lymphocytes and found to be infectious, indicating that virus was being produced in the cells and could be passaged.

These findings provide direct evidence for the infectibility of progenitors or early precursors of the myelomonocytic lineage, as was suggested by previous workers (*5, 6*). The absence of a marked cytopathic effect during acute infection corroborates work by us and others (*14*) on in vitro HIV-1 infection of promonocytic tumor cells, which are progeny of bone marrow cells of myelomonocytic lineage. In our previous study, an infected clone (U1) derived from chronically infected U-937 cells did not constitutively express virus but was inducible with phorbol myristate acetate (*14*) or cytokines (*15*) and yet showed no cytopathic effect. Recently, in vitro infection with HIV-1 of normal mature peripheral blood monocytes was achieved by a number of laboratories (*8*) and such infections revealed little or no cytopathic effect.

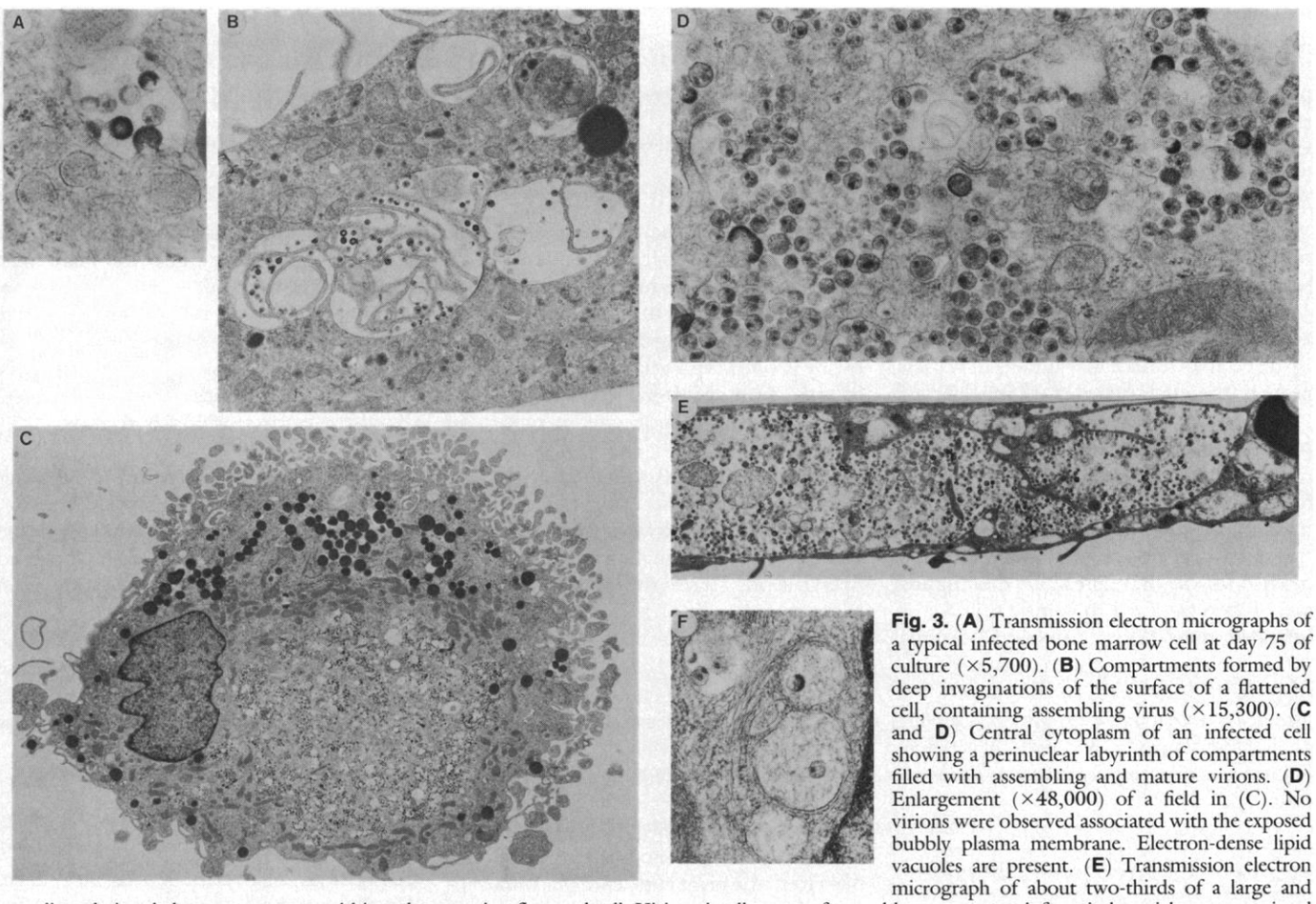

Fig. 3. (**A**) Transmission electron micrographs of a typical infected bone marrow cell at day 75 of culture (×5,700). (**B**) Compartments formed by deep invaginations of the surface of a flattened cell, containing assembling virus (×15,300). (**C** and **D**) Central cytoplasm of an infected cell showing a perinuclear labyrinth of compartments filled with assembling and mature virions. (**D**) Enlargement (×48,000) of a field in (C). No virions were observed associated with the exposed bubbly plasma membrane. Electron-dense lipid vacuoles are present. (**E**) Transmission electron micrograph of about two-thirds of a large and complicated virus-laden compartment within a degenerating flattened cell. Virions in all stages of assembly are present. A few viral particles are associated with the upper and lower cell surfaces (×12,500) (**F**) Typical virions in three granular vacuoles; one has not completed budding (×48,000).

It is difficult to detect virus in mature circulating monocytes (*16*), and virus has not been recovered from mature granulocytes. In vivo, therefore, it is likely that only a very small proportion or a subpopulation of $CD34^+$ bone marrow progenitor cells are infected with HIV-1 and the remainder differentiate normally into mature blood elements. This could explain why cytopenias of granulocyte and monocyte lineage in HIV-1 infection are relatively uncommon (*2, 3*). The extent to which infection of bone marrow progenitor cells can explain the observed hematologic abnormalities in HIV-1 infection is uncertain. The studies of Donahue *et al.* (*6*) suggest that immune-mediated response to infected progenitor cells may have a significant role in the pathogenesis of these abnormalities. Our results further indicate that in the absence of growth factors the monocytoid cells that are derived from these progenitors may persist without detectable cell division, since mitotic figures were never observed and cell numbers did not increase during the culture period of 40 to 60 days. This minimizes the likelihood of infection of a subset of $CD34^+$ cells and subsequent outgrowth of a minor infected population. Nevertheless, even if only a small proportion of precursor cells could accumulate the quantity of viral particles in vivo as was observed in our in vitro system, this could provide an important reservoir of HIV-1 and could contribute to the dissemination of virus. Furthermore, there is precedent for bone marrow serving as a reservoir for retroviral infection. In the feline leukemia virus system a latent form of the virus resides predominantly in myelomonocytic precursors of the bone marrow (*17*).

The fact that these progenitor cells could be infected with HIV-1 in the initial absence of detectable surface or intracellular CD4 protein does not rule out its involvement (*18*). Clearly, undetectable CD4 could be present and serve as the HIV-1 receptor in these cells. Alternatively, phagocytosis might serve as a mode of viral entry. The finding that CD4 develops during differentiation of these cells might also represent the mechanism of viral spread in the cultures and explain the need to culture cells for an extended time before detection of virus. More important, the gradual differentiation of these cells into cells of the monocytoid lineage is consistent with monocytes serving as viral reservoirs (*19*) and with their potential for disseminating infection to other organs.

REFERENCES AND NOTES

1. *Morb. Mortal. Wkly. Rep.* **35**, 334 (1986).
2. J. L. Spivak, B. S. Bendar, T. C. Quinn, *Am. J. Med.* 77, 224 (1984).
3. D. R. Schneider, L. J. Picker, *Am. J. Clin. Pathol.* **84**, 144 (1985).
4. F. Delacretaz, L. Perez, P. M. Schmidt, J. P. Chave, J. Costa, *Virch. Arch. A* **411**, 543 (1984).
5. M. Busch, J. Beckstead, D. Gantz, G. Vyas, *Blood* **68** (suppl. 1), 122a (1986).
6. R. E. Donahue, M. M. Johnson, L. I. Zon, S. C. Clark, J. E. Groopman, *Nature* **326**, 200 (1987).
7. D. Klatzman *et al.*, *Science* **225**, 59 (1984); M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.* **224**, 497 (1984); A. G. Dalgleish *et al.*, *Nature* **312**, 763 (1984); D. Klatzman *et al.*, *ibid.* **312**, 767 (1984); J. S. McDougal *et al.*, *J. Immunol.* **135**, 3151 (1985); J. S. McDougal *et al.*, *Science* **231**, 382 (1986).
8. D. D. Ho, T. R. Rota, M. S. Hirsch, *J. Clin. Invest.* **778**, 1712 (1986); J. K. A. Nicholson, G. D. Gross, C. S. Collaway, J. S. McDougal, *J. Immunol.* **137**, 323 (1986); S. Gartner *et al.*, *Science* **233**, 215 (1986); S. Roy and M. A. Wainberg, *J. Leuk. Biol.* **43**, 91 (1988).
9. C. I. Civin *et al.*, *J. Immunol.* **133**, 157 (1984).
10. R. G. Andrews, J. W. Singer, I. D. Bernstein, *Blood* 67, 842 (1986); L. Lue *et al.*, *J. Immunol.* **139**, 1823 (1987).
11. S. W. Kessler, D. Vembu, A. T. Black, *Blood* **70** (suppl. 1), 321a (1987).
12. S. W. Kessler, *Fed. Proc.* **46**, 1363 (1987).
13. Adherent and nonadherent, and infected and uninfected cells were prepared for TEM. Nonadherent cells were centrifuged (600*g*), fixed overnight in 2.5% glutaraldehyde in 0.1*M* cacodylate buffer, *p*H 7.4, postfixed in 1% OsO_4, block-stained in saturated uranyl acetate (UA) in 50% ethanol, dehydrated in graded ethanol and propylene oxide, and embedded in Spurr's plastic. Attached cells were processed in situ through 100% ethanol, removed from the well after adding propylene oxide, and embedded in Spurr's (method of C. Oliver, NIH, personal communication). Thin sections were stained with uranyl acetate and lead citrate and examined on a Zeiss EM 10A.
14. T. M. Folks *et al.*, *J. Immunol.* **140**, 1117 (1988); S. M. Hammer, J. W. Gillis, J. E. Groopman, R. M. Rose, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8734 (1986); P. Clapham *et al.*, *Virology* **158**, 44 (1987); C. J. Petit, F. G. Terpstra, F. Miedema, *J. Clin. Invest.* **79**, 1883 (1987); J. A. Levy *et al.*, *Virology* **147**, 441 (1985).
15. T. M. Folks *et al.*, *Science* **238**, 800 (1987).
16. S. Z. Salahuddin *et al.*, *Blood* **68**, 281 (1986); A. Ranki *et al.*, *Lancet* **ii**, 589 (1987).
17. R. G. Olsen, *Cancer Metast. Rev.* **6**, 243 (1987); J. I. Mullins, C. S. Chen, E. A. Hoover, *Nature* **319**, 333 (1986).
18. J. Sodroski, W. C. Goh, C. Rosen, K. Campbell, W. A. Hazeltine, *Nature* **322**, 470 (1986); J. D. Lifson *et al.*, *Nature* **323**, 725 (1986); J. A. Hoxie *et al.*, *Science* **234**, 1123 (1986).
19. H. E. Gendelman *et al.*, *J. Exp. Med.* **167**, 1428 (1988); J. M. Orenstein, M. S. Meltzer, T. Phipps, H. E. Gendelman, *J. Virol* **62**, 2578 (1988).
20. For above details on the cells and the purification procedures, see S. W. Kessler, in preparation.
21. R. Wiley *et al.*, *J. Virol.* **62**, 139 (1988).

22. We thank the Bone Marrow Procurement Team of the NMRI Tissue Bank and A. T. Black and D. M. Smoot for technical assistance. Supported by Naval Medical Research & Development Command Work Unit No. 63706N.M0095.003.1007. We also thank S. Honig and R. Roberts for technical assistance and B. Potts for the macrophage tropic HIV-1 strain.

9 September 1988; accepted 15 September 1988

Transcriptional Activation of a Cutinase Gene in Isolated Fungal Nuclei by Plant Cutin Monomers

GOPI K. PODILA, MARTIN B. DICKMAN, P. E. KOLATTUKUDY*

The molecular mechanism by which fungal spores that land on plants sense the contact and consequently trigger cutinase gene expression to gain entry into the plant was studied in isolated nuclei. Nuclear runoff experiments showed that the induction involved transcriptional activation. Monomers, unique to cutin, and a soluble protein factor from the fungal extract selectively activated cutinase gene transcription, probably by promoting initiation in nuclei isolated from uninduced *Fusarium solani pisi*. This in vitro transcriptional activation produced normal-sized cutinase messenger RNA. Alterations in the monomer structure diminished the transcriptional activation.

FUNGAL PATHOGENS PENETRATE THE cuticular barrier of plants by using cutinase, an extracellular enzyme produced by the germinating spore (*1*). The fungal spore senses the contact with the plant surface when the small amount of cutinase carried by the spore releases 10,16-dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid from the insoluble structural polyester of plant cuticle. These monomers, which occur in nature only as components of cutin (*2*), were found to be potent inducers of cutinase in the spores of *Fusarium solani pisi (Nectria haematococca)* (*3*). We now show that these monomers from the plant cuticle trigger transcription of a cutinase gene in the fungal pathogen. We also show that the dihydroxy fatty acid component of plant cuticle, together with a soluble protein factor from the fungus, induces selective transcription of the cutinase gene in isolated nuclei. We used this in vitro transcriptional activation as an experimental system to elucidate how a plant cuticle component is used by the pathogen as a molecular signal to sense contact with the plant and how this signal is used to transcribe the gene whose product is necessary for fungal entry into the host.

Ohio State Biotechnology Center and Department of Biochemistry, Ohio State University, Columbus, OH 43210.

*To whom correspondence should be addressed at Biotechnology Center, Ohio State University, Rightmire Hall, Columbus, OH 43210.