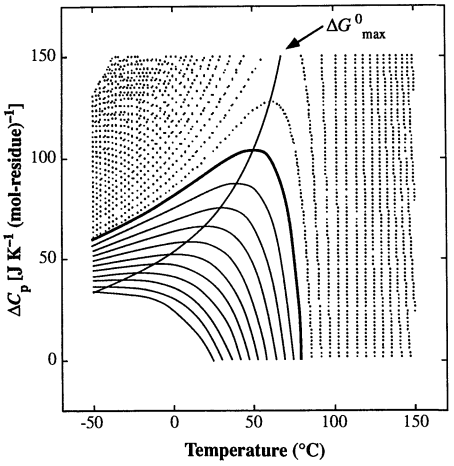

Fig. 3. Generalized map of the free energy change of protein stability per unit amino acid residue as a function of heat capacity change and temperature. The bold solid line represents the level at which $\Delta G^o/n$ is zero for the denaturation reaction of the native protein (N → D). The spacing between contour lines is 100 J mol^{-1}, with solid lines indicating positive values and dotted lines indicating negative values. The contour map was generated from Eq. 3 with ΔH^* = 6.4 kJ (mol-residue)$^{-1}$, ΔS^* = 18.1 J K^{-1} (mol-residue)$^{-1}$, and T^* = 112°C. The solid line labeled ΔG^o_{max} indicates where ΔG^o for a protein with a given ΔC_p goes through its maximum value as the temperature is raised.

J K^{-1} (mol-residue)$^{-1}$, the stability rises to a maximum value and then decreases with increasing temperature. The decrease in stability with decreasing temperature is a direct manifestation of increased hydrophobic solvation at lower temperatures and gives rise to the phenomenon of cold denaturation. At high temperatures, a decrease in stability occurs as a result of the dominance of the nonhydrophobic entropy contribution, expressed in $T\Delta S^*$. The standard state stability surface given in Fig. 3 summarizes the universal thermodynamic features found in proteins and provides a first-order representation of the thermal stability of globular proteins.

REFERENCES AND NOTES

1. P. L. Privalov, *Adv. Protein Chem.* **33**, 167 (1979).
2. J. M. Sturtevant, *Annu. Rev. Phys. Chem.* **38**, 463 (1987).
3. E. Freire and R. L. Biltonen, *Biopolymers* **17**, 463 (1978).
4. W. J. Becktel and J. A. Schellman, *ibid.* **26**, 1859 (1987).
5. F. Franks, R. H. M. Hatley, H. L. Friedman, *Biophys. Chem.* **31**, 307 (1988).
6. J. T. Edsall, *J. Am. Chem. Soc.* **57**, 1506 (1935).
7. H. S. Frank and M. W. Evans, *J. Chem. Phys.* **13**, 507 (1945).
8. S. J. Gill, S. F. Dec, G. Olofsson, I. Wadsö, *J. Phys. Chem.* **89**, 3758 (1985).
9. W. Kauzmann, *Adv. Protein Chem.* **14**, 1 (1959).
10. R. L. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8069 (1986).
11. P. L. Privalov and S. J. Gill, *Adv. Protein Chem.* **39**, 191 (1988).
12. S. J. Gill, N. F. Nichols, I. Wadsö, *J. Chem. Thermodyn.* **8**, 445 (1976).
13. J. M. Sturtevant, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2236 (1977).
14. S. J. Gill and I. Wadsö, *ibid.* **73**, 2955 (1976).
15. P. L. Privalov, *Annu. Rev. Biophys. Biophys. Chem.* **18**, 47 (1989).
16. E. Wilhelm *et al.*, *Chem. Rev.* 77, 219 (1977).
17. S. F. Dec and S. J. Gill, *J. Solution Chem.* **13**, 27 (1984).
18. H. Naghibi, S. F. Dec, S. J. Gill, *J. Phys. Chem.* **90**, 4621 (1986).
19. ———, *ibid.* **91**, 245 (1987).
20. H. Naghibi, D. W. Ownby, S. J. Gill, *J. Chem. Eng. Data* **32**, 422 (1987).
21. S. F. Dec and S. J. Gill, *J. Solution Chem.* **14**, 417 (1985).
22. G. Olofsson, A. A. Oshodj, E. Qvarnström, I. Wadsö, *J. Chem. Thermodyn.* **16**, 1041 (1984).
23. K. P. Murphy and S. J. Gill, *Thermochim. Acta* **139**, 279 (1989).
24. ———, *J. Chem. Thermodyn.* **21**, 903 (1989).
25. K. Shinoda, *J. Phys. Chem.* **81**, 1300 (1977).
26. N. Muller, *J. Solution Chem.* **17**, 661 (1988).
27. The recently highly successful continuum model for water by S. A. Rice and M. G. Sceats [*J. Phys. Chem.* **85**, 1108 (1981)] and by A. R. Henn and W. Kauzmann [*ibid.* **93**, 3770 (1989)] does not involve changes in the number of hydrogen bonds, nor has it been applied to the effect of inserting apolar molecules into water. Thus a quite different interpretation of the empirical T^* value may evolve from this model.
28. I. Prigogine and R. Defay, *Chemical Thermodynamics* (Longmans Green, London, 1954).
29. B. Lee and F. M. Richards, *J. Mol. Biol.* **55**, 379 (1971).
30. C. Chothia, *Annu. Rev. Biochem.* **53**, 537 (1984).
31. J. Bello, *Int. J. Peptide Protein Res.* **12**, 38 (1978).

32. We acknowledge helpful comments from J. Schellman and J. Edsall. Supported under NSF grant CHE 8611408.

3 October 1989; accepted 28 November 1989

Activation of Proto-Oncogenes: An Immediate Early Event in Human Cytomegalovirus Infection

ISTRAN BOLDOGH,* SAZALY ABUBAKAR, THOMAS ALBRECHT†

A rapid increase in the RNA levels of the proto-oncogenes c-*fos*, c-*jun*, and c-*myc* was detected after human cytomegalovirus infection. Neither inactivation of viral infectivity with ultraviolet irradiation (with or without psoralen), nor inhibition of translation with cycloheximide or anisomycin adversely affected the enhanced expression of proto-oncogenes, even though these treatments substantially reduced or eliminated the detection of immediate early viral antigens. The increase in the RNA levels of the proto-oncogenes was prevented in the presence of α-amanitin or actinomycin D. Thus, expression of these oncogenes appears to be induced by events occurring before the onset of viral protein synthesis, perhaps by the interaction of viral particles with the cell surface.

HUMAN CYTOMEGALOVIRUS, A VIrus with demonstrated oncogenic potential in vitro (*1*), has long been recognized for its ability to stimulate cellular DNA synthesis and cell proliferation (*2*). Infection with human cytomegalovirus (HCMV) generates cellular responses similar to those described for activation of cells by growth factors such as platelet-derived growth factor (*3*) including: increased cellular levels of the secondary messengers inositol trisphosphate (IP_3) and 1,2-diacylglycerol (DG), Ca^{2+} influx, increased cytosolic Ca^{2+} activity, and increased cellular levels of adenosine 3′,5′-monophosphate (cAMP) [reviewed in (*4*)]. It has also been demonstrated that HCMV particles that have been inactivated and appear to be incapable of giving rise to the synthesis of either immediate early (IE) or early viral antigens play an important role in the stimulation of HCMV-induced cell proliferation (*5*). These findings suggest that defective or inactivated HCMV particles have the capacity to initiate cellular replication.

The molecular interactions associated with the activation of cell proliferation have been the subject of extensive recent studies through analysis of the induction of proto-oncogenes in quiescent cells stimulated to proliferate by the addition of serum or growth factors. From the temporal pattern of their expression, it has been suggested that proto-oncogenes play a pivotal role in the regulation of cell proliferation (*6*). Since stimulation of proto-oncogene expression could be involved in cell activation by HCMV as previously described (*4*), we wished to determine whether HCMV infection affected the expression of selected proto-oncogenes. We now show that one of the consequences of HCMV infection is a striking increase in the levels of RNA derived from the proto-oncogenes c-*fos*, c-*jun*, and c-*myc*.

Quiescent human embryonic lung (LU) cells infected with HCMV demonstrated a rapid, transient increase in the levels of RNA for the proto-oncogenes c-*jun*, c-*fos*, and c-*myc* between 20 and 120 min postin-

Department of Microbiology, The University of Texas Medical Branch, Galveston, TX 77550.

*Permanent address: Department of Microbiology, Medical University of Debrecen, Debrecen H-4012, Hungary.

†To whom correspondence should be addressed.

fection when either cytoplasmic (Fig. 1A) or total (7) cellular RNAs were analyzed. Maximum stimulation of these proto-oncogenes was quantitatively similar to that achieved by serum stimulation (Table 1). During the first 120 min postinfection, the transcripts from five other proto-oncogenes (c-H-*ras*, c-*raf*, c-*erb*A1, c-*myb*, and c-*mos*) were either not detectable or did not show substantially altered levels in the total cellular RNA (Fig. 1B). These data suggest that the increases in c-*fos*, c-*jun*, and c-*myc* RNA levels were a specific response to HCMV infection.

Substantial increases in c-*fos* and c-*jun* RNA levels preceded the quantitatively similar increase in c-*myc* RNA levels. Increased levels of c-*fos* and c-*jun* RNAs were first detectable by 20 min postinfection, reached their highest level at 40 min, and by 120 min postinfection decreased to about the basal levels for quiescent, mock-infected cells (Fig. 1A). Increased levels of c-*myc* RNA were first detectable at 20 min postinfection, although a substantial increase, similar to those for c-*fos* and c-*jun* RNAs, was not observed until 40 min. Maximum levels of c-*myc* transcripts were noted at 60 min, and by 120 min they had decreased to levels approaching, but still somewhat greater, than those for the mock-infected cells. Since mock infection induced minor changes in the levels of c-*jun*, c-*fos*, and c-*myc* RNAs, the increase in specific RNAs in HCMV-infected cells was evaluated relative to the RNA levels for mock-infected cells. The maximum levels of c-*fos*, c-*jun*, and c-*myc* RNAs were 17, 11, and 7 times as great as the values for the mock-infected cells, respectively. As an internal control, we measured the RNA levels for β-actin, which remained stable from 0 to 2 hours postinfection in mock- or HCMV-infected cells (7). The increases in specific RNA species were similar when cytoplasmic and total cellular RNA were compared (7).

We used a purified HCMV inoculum in a series of experiments to exclude the possibility that contamination of our virus preparations with serum- or cell-derived growth factors contributed significantly to the observed increase in proto-oncogene expression. Extensive purification of the virus particles before their use as inocula did not reduce the increase in proto-oncogene expression (Table 1), as described for simian virus 40 (SV40) (*8*), indicating that it was unlikely that serum- or cell-derived growth factors associating with virus particles during preparation of virus stocks were substantially involved in the stimulation of proto-oncogene expression. Mild digestion of virion envelope proteins with either protease E or trypsin, however, abolished the HCMV-induced increase in the level of proto-oncogene RNAs (Table 1), suggesting that intact virions were required for stimulation of proto-oncogene expression.

The requirement for de novo protein synthesis for induction of increased proto-oncogene RNA levels by HCMV was evaluated by measuring the effect of anisomycin or cycloheximide on the amount of the specific RNA species relative to the detection of HCMV IE antigens (Table 1). Although the synthesis of IE HCMV-specific proteins was undetectable under these restrictive conditions (Table 1), the magnitude of proto-oncogene RNA levels exceeded those documented in Fig. 1, resembling the superinduction previously reported for serum-stimulated cells (*9*). The results of these experiments suggest that activation of these cellular genes is independent of the expression of IE HCMV-specific proteins and is unlikely to require de novo protein synthesis, since it has been previously shown (*9*) and confirmed in this investigation (7) that cycloheximide and anisomycin block protein synthesis in HCMV-infected cells by about 97 or 98%, respectively. The increase in proto-oncogene RNA levels was sensitive, however, to α-amanitin (Table 1), suggesting that the increase in transcripts results from the activity of RNA polymerase II. Similar results were obtained when transcription was blocked with actinomycin D (Table 1). These findings are consistent with the view that the increased levels of proto-oncogene RNAs may result from transcriptional activation of the proto-oncogenes.

To investigate further the necessity for expression of HCMV IE proteins, if any, as well as the role of virus particles in altered proto-oncogene expression, we treated the virus suspension with short-wave ultraviolet (swUV) irradiation or psoralen and long-wave ultraviolet (lwUV) irradiation to prevent viral transcription (*10*). Inactivation of the HCMV stock before infection reduced

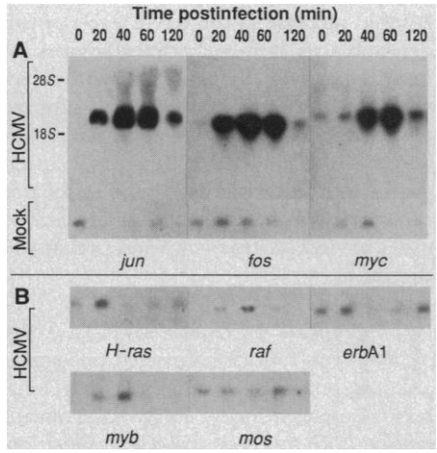

Fig. 1. The kinetics of proto-oncogene activation in LU cells after HCMV infection. (**A**) Hybridization of cytoplasmic RNA from HCMV- and mock-infected quiescent LU cells maintained in the absence of serum with ^{32}P-labeled DNA (^{32}P-DNA) probes for c-*fos*, c-*jun*, and c-*myc*. (**B**) Hybridization of total cellular RNA with ^{32}P-DNA probes for other proto-oncogenes. LU cells were grown to confluence and maintained in minimal essential medium (MEM) enriched with 8% fetal bovine serum. Afterward, the cells were arrested in serum-free MEM for 72 hours. Virus particles were pelleted from clarified stock (10,000 rpm for 20 min at 22°C) by velocity sedimentation (Beckman SW28 rotor, 27,000 rpm for 90 min at 20°C). The virus pellet was resuspended in a small amount of serum-free MEM (sedimented virus), and the suspension was added to the culture fluids of serum-arrested, precooled (4°C) cells. For further purification, the sedimented virus was pelleted through 20% (w/v) D-sorbitol in phosphate-buffered saline (PBS). The pellet was resuspended and layered on a continuous CsCl gradient (p = 1.16 to 1.36) and centrifuged at 27,000 rpm for 60 min at room temperature (*23*). The band containing enveloped virus was recovered, diluted in MEM, pelleted (27,000 rpm for 90 min at room temperature), and resuspended in serum-free MEM (purified virus). Virus was allowed to adsorb at 4°C for 30 min. For mock infection a cell lysate was prepared in the same manner as virus stock from uninfected LU cells. The HCMV- and mock-infected cultures were shifted to 37°C (0 hours postinfection) and incubated for the periods indicated. Total cellular (*24*) and cytoplasmic (*25*) RNA was isolated as described previously by others. For Northern analysis, we fractionated 14 μg of RNA per lane on 1.4% agarose (Seakem LE) after glyoxal denaturation (*26*) and transferred onto a nylon membrane (Zeta-Probe) using 10 m*M* NaOH (*27*). The blots were prehybridized and hybridized at 42°C under conditions specified by Schleicher and Schuell [50% formamide, 5× Denhardt's solution, 5× SSPE (0.15*M* NaCl, 0.01*M* NaH_2PO_4, and 0.001*M* EDTA), 0.2% SDS, and herring testis DNA (200 μg/ml)]. α-^{32}P–labeled DNA probes (specific activity = 10^9 cpm/μg) were labeled with α-^{32}P–labeled cytidine 5′-triphosphate (specific activity = 3000 Ci/mmol) by the random primer method (Boehringer Mannheim). After a 12-hour prehybridization and a 24-hour hybridization, the blots were washed twice in 6× SSPE and 0.5% SDS for 30 min at room temperature, twice in 1× SSPE and 0.5% SDS for 30 min at 37°C, and, for more stringent conditions, in 1× SSPE and 0.5% SDS for 60 min at 65°C. The blots were exposed to x-ray film for 18 hours at −20°C, and the intensity of the bands was analyzed with a densitometer. After hybridization, bands of 2.2, 2.4, and 2.6 kb were identified on autoradiograms, which correspond with the reported molecular size equivalents for c-*fos*, c-*myc*, and c-*jun* RNAs, respectively (*28*). In these experiments the following probe DNAs were used: c-*fos* (Eco RI–Bam HI), c-H-*ras* (Bam HI), c-*raf*-1 (Hind III–Eco RI), c-*erb*A1 (Eco RI), c-*myb* (Eco RI), and c-*mos* (Eco RI) fragments. The oncogene-containing plasmids were obtained from the American Type Culture Collection except that the c-*jun* (Eco RI–Pst I) oncogene was kindly provided by I. M. Verma (The Salk Institute, San Diego, California) and the v-*fos* (Eco RI), c-*myc* exon III (Eco RI), and v-*myc* (Eco RI) oncogenes were obtained from ONCOR (Gaithersburg, Maryland). The oncogene fragments were isolated by agarose (Seakem GTA) electrophoresis after restriction enzyme (Bethesda Research Laboratories) digestion.

the detection of HCMV IE antigens from >99% for cells infected with nonirradiated HCMV stock to from 17% to undetectable levels for cells infected with stock exposed to 4.8×10^{-3} to 2.9×10^{-2} J/mm^2 of swUV or psoralen and lwUV (Table 1). The increase in specific proto-oncogene RNA species was unaffected, however, at doses that reduced detection of IE antigen expression to less than 0.1% of the cells. Thus, there was no correlation between the expression of HCMV IE antigens and the increased levels of RNAs for proto-oncogenes. The increase in proto-oncogene transcripts was somewhat reduced at higher swUV doses (for example, 2.9×10^{-2} J/mm^2); it is likely that the responsible protein or proteins were affected by the swUV light. These findings suggested that the HCMV particle may be sufficient to induce the elevated proto-oncogene RNA levels in the absence of protein synthesis.

Our data suggest that the elevated levels of proto-oncogene RNAs are related to the interaction of HCMV virion components with the cell, possibly the cytoplasmic lamella, to initiate a physiological cascade associated with activation of the infected cell. This hypothesis is supported by our previous findings that HCMV particles appear to be sufficient to increase phosphatidylinositol-4,5-bisphosphate (PIP_2) hydrolysis and the cellular levels of the secondary messengers IP_3 and DG (*11*).

The proto-oncogenes induced by HCMV are thought to be involved in cellular signal transduction and regulation of cell proliferation (*6*). C-*jun* is reported to encode a transcription factor, *jun*/AP-1, which recognizes specific DNA sequences (*12, 13*). Most *jun*/AP-1 is complexed with Fos (*13*), which increases the ability of the complex to stimulate transcription. The sequence for the *jun*/AP-1 binding site—TGA N TCA (*14*)—is similar to a sequence (TGA CG TCA) located in the HCMV IE promoter-regulatory region (*15*), and identical to that reported as a cAMP-responsive element (*16*). The c-*myc* gene product is a DNA-binding protein, implicated in the rapid modulation of cellular gene expression specifically involved in the G_0/G_1 transition (*17*).

The activating effect of HCMV on proto-oncogenes could be related to disrupting normal cell functions or the initiation and progression of HCMV replication. This phenomenon may be involved in HCMV oncogenesis, perhaps through a proposed "hit and run" mechanism (*18*), as well as in the induction of HCMV-associated disease such as developmental abnormalities (*19*) and, possibly, atherosclerosis (*20*). Since in our laboratory similar findings have been obtained in another cell type (Balb/c 3T3) and for Herpes simplex virus types 1 and 2 in both LU and 3T3 cells (*7*), the induction of proto-oncogenes may be a more general phenomenon associated with viral-induced pathogenesis.

Table 1. The relation between HCMV-induced synthesis of IE antigens and elevated RNA levels for proto-oncogenes. Serum-arrested LU cells were prepared, infected, and analyzed for proto-oncogene RNA levels as described in the legend to Fig. 1. HCMV IE antigens were detected by the anticomplement immunofluorescence test (*21*). For the immunofluorescence test, LU cells were plated on glass cover slips and treated in the same way as the cells prepared for RNA analysis. Purified anti-HCMV immunoglobulin G (IgG) together with human complement and fluorescein conjugated to IgG specific for human complement C3 (Cappel) was used to detect HCMV-specific IE antigens. Protein synthesis was blocked by treating cells with anisomycin or cycloheximide continuously from 2 hours before infection through the period of maximum oncogene induction. α-Amanitin or actinomycin D was added at the time of infection. Virus suspensions were treated with either protease E (50 μg/ml) or trypsin (50 μg/ml) for 10 min at 37°C to remove possible ligands from the virus envelope (*22*). Virus stock was irradiated on an ice bed with a "germicidal" bulb producing swUV predominantly at 254 nm, at a dose rate of 8×10^{-6} J/s per square millimeter. For inactivation with psoralen and lwUV, psoralen (Sigma) was added to clarified virus suspension to obtain a final concentration of 10 μg/ml. The treated virus was collected by velocity sedimentation, resuspended in serum-free MEM, and irradiated on an ice bed with a lwUV lamp producing irradiation predominantly at 365 nm, at a dose rate of 4.2×10^{-5} J/s per square millimeter.

Extracellular stimulus	Fold increase in RNA*			Cells containing one or more HCMV IE antigens (%)
	c-*jun*	c-*fos*	c-*myc*	
Mock infection	1	1	1	<0.01
Fetal bovine serum (10%)	12	14	7	<0.01
Sedimented HCMV†	10	14	7	>99
Purified HCMV†	11	12	6	>99
Inactivated HCMV† UV (254 nm) of				
4.8×10^{-3} J/(s mm^2)	11	16	8	17
9.6×10^{-3} J/(s mm^2)	10	14	8	<0.01
1.9×10^{-2} J/(s mm^2)	9	12	7	<0.01
2.9×10^{-2} J/(s mm^2)	6	7	4	<0.01
Inactivated HCMV† psolaren and UV (365 nm) of				
2.5×10^{-2} J/(s mm^2)	10	15	8	<0.01
5.0×10^{-2} J/(s mm^2)	9	12	7	<0.01
HCMV† and				
Cycloheximide (100 μg/ml)	26	32	19	<0.01
Anisomycin (100 μg/ml)	28	29	17	<0.01
Actinomycin D (20 μg/ml)	<1	<1	<1	<0.01
α-Amanitin (4 μg/ml)	<1	<1	<1	<0.01
HCMV† treated with				
Trypsin (50 μg/ml)	<1	<1	<1	<0.01
Protease E (50 μg/ml)	<1	<1	<1	<0.01

*Measured at the time of maximum stimulation relative to the value for mock-infected cells. The data for cells treated with HCMV and cycloheximide, anisomycin, actinomycin D, or α-amanitin are relative mock-infected cells treated with the same drug and concentration. †Multiplicity: 30 plaque-forming units (PFUs) per cell (determined before any inactivation treatment).

REFERENCES AND NOTES

1. T. Albrecht and F. Rapp, *Virology* **55**, 53 (1973); L. Geder, R. Lausch, F. O'Neill, F. Rapp, *Science* **192**, 1134 (1976).
2. S. C. St. Jeor *et al.*, *J. Virol.* **13**, 353 (1974).
3. M. J. Berridge, *Biochim. Biophys. Acta* **907**, 33 (1987); D. L. Alkon and H. Rasmussen, *Science* **239**, 998 (1988).
4. T. Albrecht *et al.*, *Subcell. Biochem.* **15**, 153 (1989).
5. T. Albrecht, M. Nachtigal, S. C. St. Jeor, F. Rapp, *J. Gen. Virol.* **30**, 167 (1976); J. M. DeMarchi and A. S. Kaplan, *Virology* **82**, 93 (1977); I. Boldogh *et al.*, *Arch. Virol.* **58**, 289 (1978).
6. L. F. Lau and D. Nathans, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1182 (1987); C. W. Scheinfest, S. Fujiwara, L. F. Lau, T. P. Papas, *Mol. Cell. Biol.* **8**, 3080 (1988); R.-P. Ryseck, S. I. Hirai, M. Yaniv, R. Bravo, *Nature* **334**, 535 (1988); T. A. Owen *et al.*, *J. Biol. Chem.* **262**, 15111 (1987).
7. I. Boldogh *et al.*, unpublished data.
8. B. H. Rosenberg *et al.*, *J. Virol. Methods* **3**, 167 (1981); M. Morike, A. Quaises, D. Muller, M. Montenarh, *Oncogene* **3**, 151 (1988).
9. M. E. Greenberg, A. L. Hermanowski, E. B. Ziff, *Mol. Cell. Biol.* **6**, 1050 (1986); W. W. Lamph, P. Wamsley, P. Sassone-Corsi, I. M. Verma, *Nature* **334**, 629 (1988); R. Muller, R. Bravo, J. Burckhardt, T. Curran, *ibid.* **312**, 716 (1984).
10. D. C. Redfield, D. D. Richman, M. N. Oxman, L. H. Kronenberg, *Infect. Immun.* **32**, 1216 (1981).
11. T. Valyi-Nagy *et al.*, *Arch. Virol.* **101**, 199 (1988).
12. T. J. Bos *et al.*, *Cell* **52**, 705 (1988); P. Angel *et al.*, *Nature* **332**, 166 (1988).
13. T. Curran and R. Franza, *Cell* **55**, 395 (1988); T. D. Halazonetis, K. Georgopoulos, M. E. Greenberg, P. Leder, *ibid.*, p. 917.
14. P. Angel *et al.*, *ibid.*, p. 875.
15. G. W. Hunninghake, M. M. Monick, B. Liu, M. F. Stinski, *J. Virol.* **63**, 3026 (1989).
16. M. R. Montminy and L. M. Bilezikjian, *Nature* **328**, 175 (1987); W. J. Roester, G. R. Vandenbark, R. W. Hanson, *J. Biol. Chem.* **163**, 9063 (1988).
17. K. Alitalo *et al.*, *Nature* **306**, 274 (1983); R. Watt, A. Shatzman, M. Rosenberg, *Mol. Cell. Biol.* **5**, 448 (1985); W. H. Landschultz, P. F. Johnson, S. L.

McKnight, *Science* **240**, 1759 (1988); G. L. Prendergast and M. D. Cole, *Mol. Cell. Biol.* **9**, 124 (1989); J. Karn *et al.*, *Oncogene* **4**, 773 (1989).
18. J. C. M. Macnab, *J. Gen. Virol.* **68**, 2525 (1987).
19. S. Stagno *et al.*, *Birth Defects* **20**, 65 (1984).
20. B. L. Petrie, E. Adam, J. L. Melnick, *Prog. Med. Virol.* **35**, 21 (1988).
21. B. M. Reedman and G. Klein, *J. Natl. Cancer Inst.* **11**, 499 (1973).
22. W. H. Wunner, K. T. Reagan, H. Koprowski, *J. Virol.* **50**, 691 (1984); R. L. Epstein, M. L. Powers, R. B. Rogart, H. L. Weiner, *Virology* **133**, 46 (1984); H. P. Taylor and N. R. Cooper, *J. Virol.* **63**, 3991 (1989).
23. E.-S. Huang, S.-T. Chen, J. S. Pagano, *J. Virol.* **12**, 1473 (1973); M. Stinski, *ibid.* **19**, 594 (1976).
24. J. M. Chirgwin, A. E. Przybla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979).
25. N. M. Gough, *Anal. Biochem.* **173**, 93 (1988).
26. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
27. S. Vrati *et al.*, *Mol. Biol. Rep.* **1**, 1 (1987).
28. T. Curran, G. Peters, C. Van Beveren, N. M. Teich, I. M. Verma, *J. Virol.* **44**, 674 (1982); B. Quantin and R. Breathnach, *Nature* **334**, 538 (1988); K. Alitalo, M. Schwab, C. C. Lin, H. E. Varmus, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1707 (1983).
29. We thank J. Papaconstantinou, D. A. Konkel, J. W. Peterson, W. R. Fleischmann, Jr., and M. P. Fons for their helpful comments on a draft manuscript, and J. Middledorp for providing anti-HCMV IgG. S.A. is the recipient of a J. W. McLaughlin Research Fellowship. Supported by U.S. Environmental Protection Agency grant no. R815048.

8 September 1989; accepted 6 December 1989

Table 1. Cumulative results of HIV infection of SCID-hu mice by $HIV_{JR\text{-}CSF}$ or $HIV_{JR\text{-}FL}$ in the presence (+) or absence (−) of AZT. At 2 weeks after infection, thymic sections were analyzed by DNA PCR for the presence of human β-globin and HIV, as described in the legend to Fig. 1.

Virus strain	AZT treatment	HIV	β-globin
JR-CSF	−	34/34	34/34
JR-FL	−	6/6	6/6
JR-CSF	+	0/12	12/12
JR-FL	+	0/5	5/5

Suppression of HIV Infection in AZT-Treated SCID-hu Mice

JOSEPH M. MCCUNE,* REIKO NAMIKAWA, CHU-CHIH SHIH, LINDA RABIN, HIDETO KANESHIMA

The SCID-hu mouse, engrafted with human hematolymphoid organs, is permissive for infection with the human immunodeficiency virus (HIV). This mouse model was used to test compounds for antiviral efficacy. Two weeks after infection with HIV, 100 percent (40/40) of SCID-hu mice were positive for HIV by the polymerase chain reaction. When first treated with 3′-azido-3′-deoxythymidine (AZT), none (0/17) were HIV-positive by this assay. However, AZT-treated SCID-hu mice did have a few infected cells; after AZT treatment was stopped, viral spread was detected by polymerase chain reaction in such mice. Thus, the SCID-hu mouse provides a means to directly compare new antiviral compounds with AZT and to further improve antiviral efficacy.

MUCH HAS BEEN LEARNED ABOUT the epidemiology of the acquired immunodeficiency syndrome (AIDS) and its etiologic agent, HIV. Little is known, however, about the pathophysiology of infection in man. It has thus been difficult to evaluate prophylactic measures, such as vaccines, that might prevent infection or to establish therapies that might alter the course of established disease.

A relevant animal model for HIV infection would provide a system to evaluate the pathogenesis of AIDS and to analyze potential antiviral agents or vaccine preparations before the initiation of clinical trials. The SCID-hu mouse (*1*) was constructed precisely for this purpose. Immunodeficient C.B-17 *scid/scid* mice (*2*) are engrafted with those organs of the human hematolymphoid system that are requisite for both hematopoietic stem cell differentiation and immune function. Human hematopoietic precursor cells in the fetal liver differentiate to mature human T and myelomonocytic cells in SCID-hu mice engrafted with human fetal thymus. Implants of human fetal lymph node provide human antibody responses. Direct intrathymic or intranodal injection of HIV resulted in signs of viral replication in the SCID-hu mouse (*3*). Our data indicate that HIV infection is suppressed in SCID-hu mice that are treated with the antiviral compound, 3′-azido-3′-deoxythymidine (AZT).

Four to eight weeks after engraftment, SCID-hu mice were infected with 400 to 4000 infectious units (IU) of $HIV_{JR\text{-}CSF}$ or $HIV_{JR\text{-}FL}$ by direct intrathymic injection. This dose of virus had been shown to result in infection of 100% of SCID-hu mice by 2 weeks, as assayed by in situ hybridization (*3*). Two weeks after infection, the human thymus implants were biopsied and analyzed by the polymerase chain reaction (PCR) for the presence of HIV (Fig. 1A). PCR products for both human β-globin and for HIV were present. Cells from these biopsy specimens were also positive for immunohistochemical stains for envelope and gag epitopes (*3*). The HIV and β-globin PCR products were not detectable in biopsy specimens derived from the murine spleen (M) (Fig. 1A) or the murine lymph nodes or thymus of the same animals; nor was the HIV PCR product found in uninfected littermates kept within the same cage (Fig. 1A). Thus, direct intrathymic injection of HIV results in detectable and reproducible levels of HIV infection in the human, but not the murine, lymphoid organs of the SCID-hu mouse.

To test the possibility that such evidence of HIV infection could form the basis of an in vivo assay of antiviral efficacy, AZT was administered to SCID-hu mice with human thymic implants for 24 hours before HIV infection by intrathymic injection. These mice then received continued AZT treatment for 2 weeks. At that time, the thymus implants were biopsied for PCR analysis (Fig. 1B). After 2 weeks of AZT treatment, HIV-infected SCID-hu mice (number 5, 6, 7, and 8) had no detectable HIV-specific PCR products. This antiviral effect was reproducible with larger numbers of animals (Table 1). All (40/40) of the SCID-hu mice infected with HIV in the absence of AZT were positive by PCR after 2 weeks. None (0/17) of the SCID-hu mice treated with AZT had HIV-specific PCR products after 2 weeks. Infection was suppressed when either a T-lymphotropic isolate of HIV (JR-CSF) or a monocytotropic isolate (JR-FL) was used.

More sensitive assays were used to evaluate the qualitative nature of this antiviral effect. Thymic biopsy specimens from HIV-infected SCID-hu mice treated with AZT for 2 weeks were subjected to in situ hybridization with a ^{35}S-labeled RNA probe specific for the 3′ end of the HIV genomic transcript (Fig. 2). Fewer cells were infected in the presence of AZT and each infected cell had fewer HIV transcripts. Nonetheless, infection by HIV had occurred. Because continued rounds of viral infection might spread from such cells, SCID-hu mice that had been treated with AZT for 2 weeks and were negative for HIV (by PCR) were then switched to a regimen without AZT for an additional 4 weeks. At that time, thymic biopsy specimens were found to be positive

HIV Group, SyStemix, 3400 West Bayshore Road, Palo Alto, CA 94303.

*To whom correspondence should be addressed.