References and Notes

1. R. L. Satter, *Encyclopedia of Plant Physiology*, W. Haupt and M. E. Feinleib, Eds. (Springer-Verlag, Berlin, 1979), vol. 7, p. 442.
2. T. Sibaoka, in *Plant Growth Substances 1979*, F. Skoog, Ed. (Springer-Verlag, Berlin, 1980), p. 462.
3. B. S. Hill and G. P. Findlay, *Q. Rev. Biophys.* **14**, 173 (1981).
4. R. D. Allen, *Plant Physiol.* **44**, 1101 (1969).
5. K. Oda, T. Abe, K. Tabe, *Proc. 41st Meet. Bot. Soc. Jpn.* (1976), p. 210.
6. S. E. Williams and B. G. Pickard, in *Plant Growth Substances 1979*, F. Skoog, Ed. (Springer-Verlag, Berlin, 1980), p. 470.
7. W. H. Brown, *Am. J. Bot.* **3**, 68 (1916).
8. K. Kondo, *Jpn. Minist. Educ. Integrated Stud. Grant Rep. 336026 Ser. A* (1980).
9. R. E. Cleland, in *Plant Growth Substances 1979*, F. Skoog, Ed. (Springer-Verlag, Berlin, 1980), p. 71.
10. R. M. Spanswick, *Annu. Rev. Plant Physiol.* **32**, 267 (1981).
11. M. J. Jaffe, *Plant Physiol.* **51**, 17 (1973).
12. O. Stuhlman, *Bull. Torrey Bot. Club.* **75**, 22 (1948).
13. S. E. Williams, *Proc. Am. Philos. Soc.* **120**, 187 (1976).
14. M. T. Tyree and H. T. Hammel, *J. Exp. Bot.* **23**, 267 (1972).
15. Y. Yamamoto, R. Yamamoto, Y. Masuda, *Plant Cell Physiol.* **15**, 833 (1974).
16. J. Ashida, *Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B9* (1934), p. 141.
17. D. Keifer and R. M. Spanswick, *Plant Physiol.* **64**, 165 (1979).

18. We would like to thank R. M. Spanswick for the use of his laboratory facilities and to acknowledge support from National Science Foundation grant PCM 78-12119 to R. M. Spanswick.

29 July 1982; revised 9 September 1982

New Method for Detecting Cellular Transforming Genes

Abstract. *Tumor induction in athymic nude mice can be used to detect dominant transforming genes in cellular DNA. Mouse NIH 3T3 cells freshly transfected with either cloned Moloney sarcoma proviral DNA or cellular DNA's derived from virally transformed cells induced tumors when injected into athymic nu/nu mice. Tumors were also induced by cells transfected with DNA from two tumor-derived and one chemically transformed human cell lines. The mouse tumors induced by human cell line DNA's contained human DNA sequences, and DNA derived from these tumors was capable of inducing both tumors and foci on subsequent transfection. Tumor induction in nude mice represents a useful new method for the detection and selection of cells transformed by cellular oncogenes.*

Recent advances in the technique of DNA-mediated gene transfer have led to the identification (*1–10*) and molecular cloning (*11–13*) of dominant human transforming genes from neoplastic cells. In most cases the genes have been detected by means of focus forming assay on NIH 3T3 cells although in one study the genes were also detected by examining the ability of transformed cells to grow in soft agar (*2*). Since the identification of foci is dependent on cell culture conditions and cell morphology and since the latter can represent a somewhat subjective measure of cell transformation, we sought to develop an alternative screening and selection method for transformed cells. Transformed cells can induce tumors in mice, and in particular, tumorigenicity in athymic nu/nu mice has been used to definitively demonstrate the transformed phenotype (*14*). The potential of the nude mouse as a vehicle for detecting and isolating transformed cells from a mass population of DNA transfected cells is described in this report.

We initially measured the ability of cells transfected with various amounts of the molecularly cloned acute transforming provirus of Moloney murine sarcoma virus (MSV) (*15*) to induce tumors in nude mice (Fig. 1). The results show that mice injected with MSV DNA-transfected cells developed tumors which appeared between 6 to 8 weeks after injection. At the lowest level tested (0.4 ng), one of three mice developed a palpable tumor, while at the highest level tested (400 ng), four of five mice developed tumors. A portion of each transfected cell population was also assayed in the conventional focus induction assay, and transformed foci were counted 14 days later. One focus was observed on cells treated with 0.4 ng of MSV DNA, while cells treated with 400 ng gave approximately 400 foci. It is unclear why only four out of five mice receiving cells transfected with 400 ng of MSV DNA developed tumors, although it may be due to the immune response to tumor cells present in nude mice (*16*). However, the fact that tumors were observed when cells had been transfected with as little as 0.4 ng of MSV DNA indicated that the tumor assay was highly sensitive and able to detect the presence of small numbers of transformed cells. The tumors all arose at the site of injection, grew rapidly, and appeared to be undifferentiated fibrosarcomas (*17*). Superinfection of cell lines derived from tumors with Moloney murine leukemia virus (MuLV) resulted in the rescue of infectious transforming virus from 50 percent of these cultures (four rescued of eight tested). Similar proportions of MSV foci induced by conventional DNA transfection assays are rescuable with MuLV (*18*). Mice injected with untransformed cells or cells transfected with carrier calf thymus DNA did not develop tumors during the 15-week observation period.

We also examined the ability of cells transfected with cellular DNA from virally transformed cells to induce tumors in mice. DNA was isolated from HTMF cells, an MSV-transformed mink cell line containing a single copy of MSV (*15*), and SV80 cells, an SV40-transformed human fibroblast cell line (*19*). These cellular DNA preparations induced tumors in nude mice in 5 to 7 weeks (Table 1), and foci were observed in portions of the HTMF DNA-transfected cells maintained in tissue culture. MSV could be rescued from two of three tumors induced by HTMF DNA-transfected cells. Three tumors induced by SV80 DNA transfection were explanted, and the resulting cell lines were tested for SV40 T antigen expression in an immunofluorescence assay (*20*). From 40 to 80 percent of the cells present in each tumor-derived cell line exhibited nuclear staining characteristics of SV40-transformed cells. These results showed that the assay detected single-copy transforming genes present in cellular DNA and suggested that that assay could be used to detect such sequences in DNA isolated from transformed human cells.

All of the tumors observed in the experiments described above appeared in 5 to 8 weeks, and no tumors were observed in control experiments. However, in experiments not shown, we found that tumors could be induced in mice injected with NIH 3T3 cells transfected with DNA from nonneoplastic tissues, including calf thymus, human placenta, and mouse NIH 3T3 cells. The tumors derived from human placenta DNA transfections lacked detectable human repetitive sequences when analyzed as described below. These tumors developed later (8 to 15 weeks) than tumors induced by cells that had been transfected with DNA containing dominant transforming genes. In addition, the frequency of tumor formation in these experiments was greater when large numbers of NIH 3T3 cells (5×10^6 to 1×10^7 per mouse) were injected and was also influenced by the length of time the cells were maintained in culture prior to injection. Consequently, the incidence of these tumors can be reduced by injecting $< 2 \times 10^6$ cells into each mouse 1 to 5 days after transfection. Cells to be transfected are used either directly after revival from frozen stocks or are passaged no more than twice at subconfluent densities. In addition, our experiences indicate that tumors arising more than 9 weeks after the injection of transfected

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Table 1. Transforming efficiencies of cellular DNA from transformed cells and tumor-derived cell lines.

Cell line	Origin	Foci*	Tumors†	Tumor appearance‡ (weeks)	Human sequences§
HTMF	MSV-transformed mink fibroblast (*15*)	0.1	5/14	6 to 7	NT‖
SV80	SV40-transformed human fibroblast (*18*)	NT	3/5	5	NT
A1165	Human pancreatic carcinoma (*23*)	0.35 to 0.67	1/5	6	1/1
HT1080	Human fibrosarcoma (CCL 121) (*24*)	<0.01¶	5/5	5 to 9	2/5
MNNG-HOS	Chemically transformed human osteosarcoma (CRL 1574) (*25*)	0.02 to 0.11	3/5	5	3/3

*Transfections were completed as described (*18*) by adding 20 μg DNA to each of five plates of NIH 3T3 cells, and transformation of NIH 3T3 cells was assessed either by injecting 1×10^6 to 5×10^6 of the transfected cells into nude mice or by maintaining the transfected cells in tissue culture and monitoring the appearance of foci (see legend to Fig. 1). The average number of foci per microgram of DNA is indicated. †Number of tumors observed/number of mice injected. ‡Range of weeks after injection in which most tumors developed. §The presence of human sequences in tumors was determined by blot hybridization using Blur 8 plasmid as a probe (number of tumors containing human sequences/number examined; see legend to Fig. 2). ‖NT, not tested. ¶Indicates no foci were detected in cells transfected with 100 μg of DNA.

cells are unlikely to contain detectable transfected DNA.

The sensitivity of this assay in detecting dominant transforming genes in the DNA of tumor cell lines was tested with high molecular weight DNA prepared from human cell lines (Table 1). Cells transfected with DNA prepared from a human pancreatic carcinoma line (A1165), a human fibrosarcoma line (HT1080), and a chemically transformed human osteosarcoma line (MNNG-HOS) induced tumors in nude mice in 5 to 8 weeks, suggesting that these cell lines contained active transforming genes. To determine whether human sequences were present in these tumors, DNA derived from the tumors and from tumor cell line explants were examined for the presence of the Alu family of highly repeated human sequences. This method has been utilized (*7, 8, 10–12*) to detect transfected human DNA sequences in transformed NIH 3T3 cells. Our analysis with Eco RI digested DNA from a tumor induced by the human pancreatic carcinoma DNA is shown in lane 1, Fig. 2A, and is compared to a nude mouse transplant of the same tumor (Fig. 2A, lane 2). Essentially identical Alu probe hybridization patterns were observed in both DNA preparations, showing that the bulk of the newly acquired human sequences were stable during propagation in nude mice. Figure 2B shows Alu probe hybridization patterns observed in the Eco RI digested DNA of tumors induced by DNA from a chemically transformed human osteosarcoma cell line (lanes 4 to 7) and a human fibrosarcoma cell line (lanes 8 to 11). With the exception of the tumor DNA analyzed in lane 11 and two others (not shown), all tumor DNA samples were clearly positive for human Alu sequences by comparison to the NIH 3T3 control (Fig. 2B, lane 1).

To test the hypothesis that the acquired human sequences were responsible for the tumor induction, DNA prepared from the mouse tumors was used to induce secondary foci and tumors. DNA from the primary and transplanted nude mouse tumor induced by human pancreatic carcinoma cell DNA described above induced foci with efficiencies which ranged from 0.1 to 5.0 FFU/μg (FFU, focus-forming units). Both DNA preparations also induced tumors in nude mice. In five experiments tumors arose in 12 of 28 mice from 3 to 7 weeks after injection.

DNA from the MNNG-HOS derived tumor (Fig. 2B, lane 4) also induced foci (0.05 FFU/μg) and tumors (four of ten mice, 5 weeks after injection). DNA from several secondary foci and tumors were digested with Eco RI and analyzed for human Alu family DNA sequences. The results show that Eco RI digested DNA from independently derived foci induced by the pancreatic carcinoma primary tumor contained several Alu-containing DNA fragments of similar size, suggesting that transformation may be due to the presence of a specific human DNA sequence (results not shown). Two secondary tumors also contained human DNA sequences. Surprisingly, the secondary tumors hybridized more extensively with the Alu probe than did the primary tumor. This may result from amplification of the transfected DNA in the recipient NIH 3T3 cell and has been observed by others (*8*) (see for example, lane 3, Fig. 2A).

For purposes of comparison to the nude mouse assay, DNA prepared directly from the human pancreatic carci-

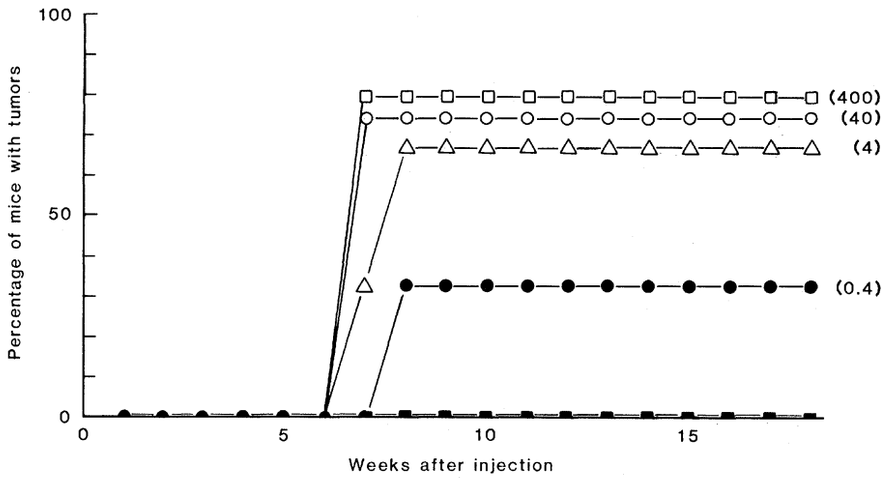

Fig. 1. Tumor induction in BALB/c nude mice by NIH 3T3 cells transfected with various amounts of cloned MSV proviral DNA. NIH 3T3 cells seeded in 60-mm culture dishes were transfected with mixtures of Eco RI digested λm1 MSV DNA (*15*) (0.4 to 400 ng) and carrier calf thymus DNA (24 μg) as described (*18*). Five replicate plates were transfected at each λm1 MSV DNA level. After 24 hours, cells were removed from the culture dishes with trypsin-EDTA and suspended in complete growth medium. Cells from each set of five plates were pooled, washed twice with 10 ml of serum-free Dulbecco's modified medium, and suspended in 0.5 ml of serum-free medium. Groups of five nu/nu (BALB/c) weanling mice were injected with the cell suspension (0.1 ml each) subcutaneously, and the mice were monitored for tumor development. Portions of some of the tumors were established as tissue culture lines, and the remaining tumor material was frozen at −70°C until DNA was prepared. The number of cells injected into each mouse, and the amount of cloned λm1 MSV DNA (shown in parentheses) transfected onto each dish are: □, 8×10^5 cells, 400 ng; ○, 7×10^5 cells, 40 ng; △, 3×10^5 cells, 4 ng; ●, 4×10^5 cells, 0.4 ng; ■, 8×10^5 cells, no MSV.

noma, human fibrosarcoma, and chemically transformed human osteosarcoma cell lines was tested in a conventional transfection focus assay. No foci were observed with the fibrosarcoma cell DNA, while two Alu positive tumors were induced. This could indicate that the nude mouse assay may detect or select for some transformation events which are not detected in conventional focus assays. However, others have reported a low level of focus-forming activity for DNA isolated from this cell line (*12*). DNA from the MNNG chemically transformed cell line induced foci with an efficiency of 0.02 to 0.11 FFU/μg of DNA and the foci contained human DNA sequences (Fig. 2B, lanes 2 and 3). DNA from the human pancreatic carcinoma line induced foci with efficiencies of 0.35 to 0.67 FFU/μg, an indication that, for this transforming gene, the focus assay is, perhaps, the more sensitive method. Therefore, the use of the nude mouse assay together with the conventional focus assay may provide additional sensitivity for detecting human transforming genes. Obviously, the sensitivity of the nude mouse assay may be improved by injecting more mice per experiment.

Tumors were also induced in mice by cells that were transfected with DNA from other cell lines derived from human neoplasms, but the tumors appeared late (9 to 15 weeks after injection) and those examined did not contain detectable human Alu DNA sequences (*20*). These lines include a human melanoma line (A1589), a human bladder carcinoma line (A1663), a human teratocarcinoma line (Hu Tera 1) (*21*), and a chemically transformed human fibroblast line (HUT 14) (*22*).

Our data suggest that the induction of tumors by newly transfected NIH 3T3 cells represents a powerful system for the selection of transformed cells. This method obviates the difficulties associated with monitoring and maintaining confluent, morphologically normal monolayers in tissue culture for many weeks and eliminates the time-consuming microscopic screening of large numbers of tissue culture dishes. Tumors that arise in nude mice are readily identified. Both DNA and RNA can be isolated directly from mouse tumors to screen for the presence and expression of transfected sequences. All tumors examined have been readily transplantable in nude mice and can be easily explanted into tissue culture. Our assay should provide a convenient alternative method for detecting tumor-transforming genes and may help further increase the number of human transforming sequences that can be identified and analyzed.

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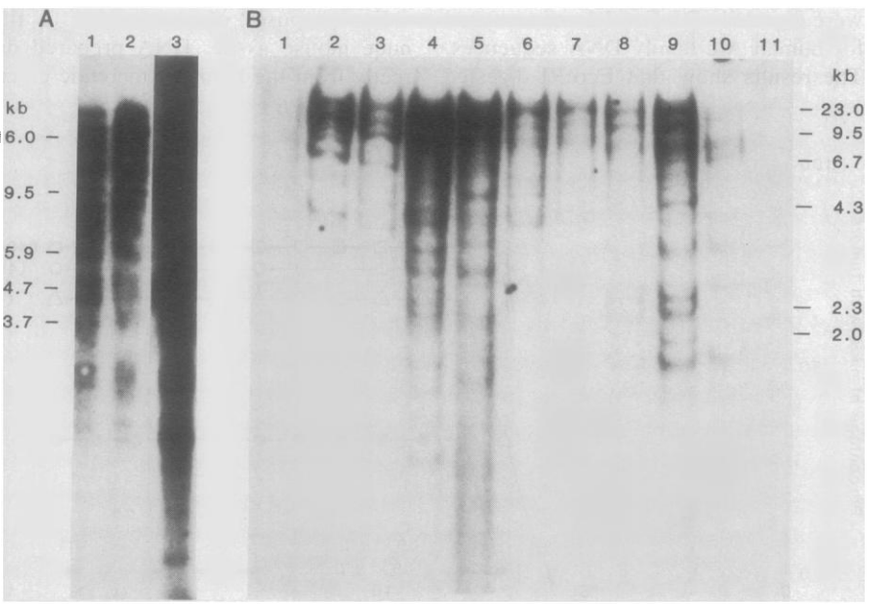

Fig. 2. The presence of human DNA sequences in tumors, tumor-derived cell lines, and transformed NIH 3T3 foci. Tumors and foci were produced in experiments in which NIH 3T3 cells were transfected with DNA from human tumor cell lines (Table 1). DNA (10 μg) extracted as described (*18*) from tumor cell lines was digested with Eco RI and subjected to electrophoresis in 0.7 percent agarose gels. It was transferred to nitrocellulose filters (*26*) and hybridized with Blur 8 plasmid DNA probe (*27*), ^{32}P-labeled by nick translation (*28*). (A) Analysis of the DNA preparations: (lane 1) a tumor induced by DNA from A1165, a pancreatic carcinoma cell line; (lane 2) a nude mouse viral transplant of the tumor analyzed in lane 1; (lane 3) secondary tumor from A1165 induced with DNA from the tumor analyzed in lane 1. (B) (lane 1) Untransformed NIH 3T3 cells; (lanes 2 and 3) primary foci induced by DNA from MNNG-HOS, a chemically transformed human cell line; (lanes 4, 5, and 7) three tumors induced by DNA from MNNG-HOS; (lane 6) a cell line obtained from the tumor analyzed in lane 5; (lane 8) a cell line derived from the tumor analyzed in lane 9; (lanes 9 to 11) tumors induced by DNA from a human fibrosarcoma cell line, HT1080. The positions of migration and size in kilobases of Eco RI–Bam HI digested (A) and Hind III digested (B) λ phage markers are indicated.

References and Notes

1. C. Shih, B. Z. Shilo, M. P. Goldfarb, A. Dannenberg, R. A. Weinberg, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5714 (1979).
2. G. M. Cooper, S. Okenquist, L. Silverman, *Nature (London)* **284**, 418 (1980).
3. G. M. Cooper and P. E. Neiman, *ibid.* **287**, 656 (1980).
4. T. G. Krontiris and G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1181 (1981).
5. C. Shih, L. C. Padhy, M. Murray, R. A. Weinberg, *Nature (London)* **290**, 261 (1981).
6. M. A. Lane, A. Sainten, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5185 (1981).
7. M. J. Murray, B. Z. Shilo, C. Shih, D. Cowing, H. W. Hsu, R. A. Weinberg, *Cell* **25**, 355 (1981).
8. M. Perucho, M. Goldfarb, K. Shimizu, C. Lama, J. Fogh, M. Wigler, *ibid.* **27**, 467 (1981).
9. N. Hopkins, P. Besmer, A. B. DeLeo, L. W. Law, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7555 (1981).
10. M. A. Lane, A. Sainten, G. M. Cooper, *Cell* **28**, 873 (1982).
11. C. Shih and R. A. Weinberg, *ibid.* **29**, 161 (1982).
12. S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, K. C. Robbins, M. Barbacid, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2845 (1982).
13. M. Goldfarb, K. Shimizu, M. Perucho, M. Wigler, *Nature (London)* **296**, 404 (1982).
14. J. Fogh and B. C. Giovanella, Eds., *The Nude Mouse in Experimental and Clinical Research* (Academic Press, New York, 1978).
15. G. F. Vande Woude, M. Oskarsson, L. W. Enquist, S. Nomura, M. Sullivan, P. J. Fischinger, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4464 (1979).
16. R. B. Heberman, M. E. Numa, D. H. Lanvin, *Int. J. Cancer* **16**, 216 (1975); B. C. Giovanella, J. S. Stehlin, L. J. Williams, *Proceedings of the International Workshop Nude Mice, 2nd, 1976* (1977), pp. 475–482; B. Sordat, C. Merenda, S. Carrel, *ibid.*, pp. 313–325.
17. K. Stromberg, personal communication.
18. D. G. Blair, W. L. McClements, M. Oskarsson, P. J. Fischinger, G. F. Vande Woude, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3504 (1980).
19. G. J. Todaro and C. Meyer, *J. Natl. Cancer Inst.* **52**, 167 (1974).
20. Serums were obtained from the Office of Program Resources and Logistics, National Cancer Institute.
21. J. Fogh and G. Trempe, in *Human Tumors in*

vitro, J. Fogh, Ed. (Plenum, New York, 1975), p. 115.
22. T. Kakunaga, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1334 (1978).
23. M. Lieber, J. Mazzetta, W. Nelson-Rees, M. Kaplan, G. Todaro, *Int. J. Cancer* **15**, 741 (1975).
24. S. Rasheed, W. A. Nelson-Rees, E. M. Toth, P. Arnstein, M. B. Gardner, *Cancer* **33**, 1027 (1979).
25. J. S. Rhim, D. K. Park, P. Arnstein, R. J. Huebner, E. K. Weisburger, W. A. Nelson-Rees, *Nature (London)* **256**, 751 (1975).
26. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
27. W. R. Jelinek, T. P. Toomey, L. Leinwand, C. H. Duncan, P. A. Biro, P. V. Choudary, S. M. Weissman, C. M. Rubin, C. M. Houck, P. L. Deininger, C. W. Schmid, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1398 (1980).
28. T. Maniatis, A. Jeffrey, D. G. Kleid, *ibid.* **72**, 1184 (1975).
29. These experiments were performed under containment levels specified by the NIH Guidelines. We thank J. DeLarco, J. S. Rhim, T. Kakunaga, and P. J. Fischinger for providing transformed human cell lines used in these studies; and S. Showalter, J. Dunn, and Karen Cannon for technical assistance.

25 August 1982; revised 5 October 1982

Virus-Induced Alterations in Homeostasis: Alterations in Differentiated Functions of Infected Cells in vivo

Abstract. *The noncytopathic lymphocytic choriomeningitis virus displays a tropism for the anterior lobe of the murine pituitary gland. Virus replicates in cells that make growth hormone. This results in a diminished synthesis of growth hormone with a concomitant clinical picture of retarded growth and hypoglycemia. However, there is no morphologic evidence of either cell necrosis or inflammation in the anterior lobe of the pituitary. Hence, during infection in vivo, a noncytopathic virus may turn off the "differentiation" or "luxury" function of a cell while not killing that cell (loss of vital function). This in turn can disrupt homeostasis and cause disease. This model illustrates a novel way whereby viruses may cause disease.*

During the course of infection, viruses injure cells by two distinct mechanisms. The first is a lethal attack by toxic products of the viral genome or disruption of regulatory (vital) functions needed for the cells' survival. Thus, by virtue of its cytopathic properties, the virus itself directly destroys the cell (*1*). The second mechanism is indirect and occurs with noncytopathic as well as cytopathic viruses. Here, the virus produces antigens foreign to the host or alters host antigens on cell surfaces. Such cells, when recognized by the host's immune system, are killed (*2*). The end product of both mechanisms is similar and provides a well-recognized morphologic picture of cell destruction in vivo, usually accompanied by inflammatory cell infiltrate characteristic of virus infections (*2, 3*). In contrast, some viruses or variants that arise in vivo can cause a noncytopathic persistent infection (*4*). Studies in vitro indicate that such virus-infected cells can have normal morphologic appearance, growth, and cloning rates, but demonstrate dysfunctions in their differentiated (luxury) cell function (*5*).

The immediate goal of the study described here was to determine whether a virus could persist in vivo in cells with specialized or differentiated functions and by the process of infection alter these functions but not kill the cell. As we show here, a relatively noncytopathic virus, lymphocytic choriomeningitis virus (LCMV), can infect cells of the anterior lobe of the pituitary gland that normally make growth hormone (GH). Morphologically these infected cells appear normal. Further, the pituitary tissue offers no evidence of either cell lysis or an inflammatory infiltrate. Most important, the production of GH by these cells diminishes significantly, and the result is a marked impairment in growth of the infected mouse.

Newborn C3H/St mice were inoculated with 60 plaque-forming units (PFU) of LCMV Armstrong strain 1371 (*2, 5*) cloned three times (clone 3B). Control, matched litters were either inoculated with medium (virus diluent) or not inoculated. The mice were weighed when they were 3, 6, 16, and 20 to 21 days of age. [Most C3H/St mice (> 99 percent) die by 30 days of age (*6*). The cause of death is uncertain, but is believed to be metabolic; a histopathologic survey of cells from organs performing vital functions is essentially normal (*6*).] Randomly selected representatives of LCMV infected and control groups were killed when they were 3, 6, or 16 days old, and their pituitary glands were removed for immunohistochemical, virological, biochemical, and morphological studies.

To locate viral antigens and GH we used pituitary glands that had been quick-frozen in liquid nitrogen, cut into 4-μm sections, and fixed. We examined these sections by immunofluorescence techniques (*2, 5*). The viral antigens we sought were the three known structural polypeptides of LCMV: two glycoproteins that insert into the cell's surface and one nucleoprotein found only in the cytoplasm of infected cells (*7*). Reagents to identify these components were murine monoclonal antibodies to LCMV, its two glycoproteins, and the nucleoprotein (*8*); others were monospecific monkey antibody to murine GH (*9*), goat antibody to murine immunoglobulin G (IgG) conjugated to either fluorescein isothiocyanate or rhodamine, and rabbit antibody to human IgG conjugated to fluorescein isothiocyanate (*10*).

To identify GH-producing cells by light or electron microscopy, we used the pituitaries of mice that had been exsanguinated by cardiac puncture and perfused with 3 percent glutaraldehyde (*11*). In several instances, thin sections of the pituitaries on gold grids were reacted with monkey antibody to murine GH, and then with goat antibody to monkey IgG conjugated to 8 to 10 nm gold particles (*12*).

Figure 1 shows that LCMV antigens were localized in the cells of the pituitary gland's anterior lobe. It also shows the corresponding clinical picture of an undersized virus-infected mouse. All of the 25 virus-infected mice were undersized at 16 days. The mean weight of the infected mice (± standard deviation) was

Fig. 1. (A) A 16-day-old C3H/St mouse persistently infected at birth with LCMV (bottom) and an uninfected littermate control (top). (B) Demonstration of viral antigen in the cells of the anterior pituitary of 16-day-old C3H/St mouse persistently infected with LCMV. The frozen section (4 μm) was reacted with monoclonal murine antibody to LCMV nucleoprotein and then with rhodamine-conjugated goat antibody to murine IgG. Cells of the middle and posterior lobes of the pituitary did not express viral antigens.

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