at birth before the neonates had begun to nurse. None of the 81 offspring was infected. The nine dams were killed and autopsied. All had mesocercariae in their mammary glands.

To determine whether postnatal infection could occur, we orally inoculated 11 parturient mice with 200 mesocercariae each, returned them to their litters, and allowed them to nurse the offspring. Before the females were infected, however, three neonates were removed from each litter and examined as controls. None was infected. Three uninfected, age-matched neonates from our rodent vivarium were transferred to each litter to restore its full complement. After 21 days a total of 99 nurslings from ten of the litters were killed and autopsied. All 99 were infected with mesocercariae, including the 30 transfers. Autopsies of ten of the lactating mothers showed mesocercariae in the mammary glands of each.

The eleventh female and her litter were used to determine whether initial infection of a paratenic host could pass to a second litter and then to a third generation. The female was mated again, and she produced a second litter of ten offspring. At 21 days postpartum the offspring were killed and autopsied. All were infected with mesocercariae. Females of the first litter were reared to maturity and mated. Only two produced litters. Autopsies of the 17 third-generation offspring 21 days postpartum showed 13 to be infected with mesocercariae.

The murine paratenic host was similar to the feline definitive host in that transmammary infection occurred in 100 percent of the second generation that nursed from infected mothers. Also, females of both species were able to infect a second litter. The two host species differed significantly in that only paratenic hosts could transmit an initial infection to the third generation. Mesocercariae undergo a complex migration in the definitive host, with diversion to the mammary glands occurring only in lactating females. In a paratenic host mesocercariae behave as though it were perpetually lactating, and in each generation they migrate to the mammary glands, there to remain, without further development, until being passed to the next generation.

Of the two mammalian species we studied, the mouse most closely models the larval dynamics in a human. This is important from an epidemiological point of view because mesocercarial stages are highly pathogenic in humans. If our results in mice are extrapolated to humans, then one may reasonably surmise that a human female, once infected, will transmit mesocercariae to all the children she nurses. Moreover, her female offspring may pass the same worms to their offspring. These migrating larvae might invade vital organs of a newborn, leading to physical or mental impairment, or even death.

WESLEY L. SHOOP
KENNETH C. CORKUM
Department of Zoology and Physiology, Louisiana State University, Baton Rouge 70803

References and Notes

1. M. Shea, A. L. Maberley, J. Walters, R. S. Freeman, A. M. Fallis, *Trans. Am. Acad. Ophthalmol. Otolaryngol.* **77**, 784 (1973).
2. R. S. Freeman *et. al.*, *Am. J. Trop. Med. Hyg.* **25**, 803 (1976).
3. P. C. Beaver *et al.*, *ibid.* **26**, 422 (1977).
4. W. M. Stone and F. W. Smith, *Exp. Parasitol.* **34**, 306 (1973).
5. J. B. Rees, W. E. Sykes, M. D. Rickard, *Aust. Vet. J.* **51**, 497 (1975).
6. G. C. Miller, *Parasitology* **82**, 335 (1981).
7. W. L. Shoop and K. C. Corkum, *J. Parasitol.* **69**, 731 (1983).
8. A. D. Johnson, *ibid.* **54**, 324 (1968).
9. W. L. Shoop and K. C. Corkum, *ibid.*, in press.
10. ———, *ibid.* **67**, 928 (1981).

5 December 1983; accepted 10 January 1984

Simian AIDS: Isolation of a Type D Retrovirus and Transmission of the Disease

Abstract. *A type D retrovirus related to but distinct from Mason-Pfizer monkey virus was isolated in vitro from the blood of two rhesus monkeys (*Macaca mulatta*) with simian acquired immunodeficiency syndrome (SAIDS). Three juvenile rhesus monkeys that were injected intravenously with tissue culture fluids containing this virus developed SAIDS after 2 to 4 weeks.*

The simian acquired immunodeficiency syndrome (SAIDS), which occurs endemically in colonies of macaque monkeys in the United States (*1, 2*), resembles the acquired immunodeficiency syndrome (AIDS) in humans in overall clinical manifestations, pathology, and immune deficiency. However, in the simian form of the disease, the ratio of helper to suppressor T cells is not reversed, nor is there a high incidence of *Pneumocystis carinii* pneumonia in affected monkeys. By means of a filterable agent present in tissue extracts and plasma of sick monkeys, SAIDS has been transmitted to healthy monkeys (*2, 3*). Here we report the isolation of a new type D retrovirus resembling Mason-Pfizer monkey virus (MPMV) from the blood of two rhesus monkeys (*Macaca mulatta*) with SAIDS. The virus was grown in tissue culture, and tissue culture fluids were used to transmit SAIDS to juvenile rhesus monkeys.

In a study of some of the biophysical properties of the then unidentified etiologic agent, we obtained plasma from an infected monkey (RM-20265) and mixed it in the cold (for 20 minutes) with an equal volume of ether to destroy the infectivity of ether-sensitive agents. Untreated plasma and ether-treated plasma were each inoculated into juvenile rhesus monkeys. After 6 months, the two animals that received ether-treated plasma remained healthy whereas the four animals that received untreated plasma developed SAIDS as defined previously (*1, 4*). Plasma from a rhesus monkey with SAIDS was subjected to ultracentrifugation, and the pellet, after being suspended in phosphate-buffered saline, was centrifuged to equilibrium in a 20 to 60 percent linear sucrose gradient. The gradient was divided into six fractions, and each fraction was inoculated intravenously into rhesus monkeys. Only the lightest two fractions (1.14 to 1.18 g/ml average density) transmitted SAIDS to recipient monkeys. These data indicated that the SAIDS agent had the physical properties of an enveloped virus.

The studies that led to the isolation of the SAIDS agent are summarized in Fig. 1. RM-18610 was a 3-year-old female with spontaneously occurring SAIDS (*5*). A mixture of tissue homogenates from RM-18610 was inoculated into RM-B883, who developed SAIDS 4 months later. A heparinized blood sample from RM-B883 was inoculated intravenously into two 12-month-old monkeys (RM-20141, RM-20335) and into primary rhesus monkey kidney (Rh-MK) cells (culture C1132). RM-20141 and RM-20335 had an accelerated course of the disease and were moribund from SAIDS by 60 and 65 days after inoculation. Their symptoms were typical, and included generalized lymphadenopathy, splenomegaly, neutropenia, diarrhea, weight loss, and lymphoid depletion (*1–5*).

Cultures C1132 and C1281 (the latter being inoculated with blood from a different SAIDS case, RM-20265) were selected for further study because the blood used to infect these cultures had produced SAIDS in juvenile rhesus monkeys (*3*). Cells from the two cultures were passaged three times and a portion

was prepared for electron microscopy and for hemadsorption assays. Some cells were stored in liquid nitrogen and their culture media were frozen at −70°C. C1132 cells displayed no cytopathic effect, but a few syncytia were noted. The culture was negative in the hemadsorption assay against the red cells of eight species (rhesus monkey, human, chicken, rabbit, guinea pig, goose, rat, and rattlesnake). C1281 cells also displayed a few syncytia. C1281, and subsequently C1132, when examined by electron microscopy, had particles resembling type D retroviruses ranging in size from 110 to 130 nm (Fig. 2). Occasional budding particles, characteristic precursors of type D virions, were seen (*6*, *7*). Uninfected Rh-MK cells showed no virus particles.

For transmission studies in vivo (Fig. 1), 4.0 ml of frozen medium from C1132 was thawed and inoculated intravenously into RM-21308, an 18-month-old female. This animal developed neutropenia, generalized lymphadenopathy, and splenomegaly after 16 days and by 30 days after inoculation had a decreased lymphocyte response to the mitogens concanavalin A, phytohemagglutinin, and pokeweed mitogen. At 52 days, the moribund animal was killed. Lymphoid tissue sections showed lymphoid depletion typical of SAIDS (*1*, *4*). Two juvenile monkeys inoculated with comparable amounts of uninfected Rh-MK culture fluids still remain healthy 5 months after inoculation.

To confirm this observation, we subjected medium from C1132 to ultracentrifugation (50,000*g*) and, resuspended the pellet in fresh culture medium. Portions of this suspension were inoculated onto fresh Rh-MK cells (C1055). After two passages, 120 ml of the C1055 medium was subjected to ultracentrifugation, the pellet was resuspended in culture medium, and portions of the suspension were inoculated intravenously, without freezing, into two juvenile females (RM-21315 and RM-21345). These monkeys developed, by 16 days after inoculation, generalized lymphadenopathy, splenomegaly, and neutropenia, and their peripheral lymphocytes showed a decreased mitogenic response. Lymph nodes biopsied on days 16 and 23 showed changes typical of SAIDS. These two animals are still alive with SAIDS 8 weeks after inoculation.

No sex differences in susceptibility to SAIDS have been observed (*1*–*5*). In the present studies, two juvenile male monkeys (1 to 2 years old) inoculated with culture fluids from C1055 have developed the early signs of SAIDS 4 weeks after inoculation (generalized lymphadenopathy and splenomegaly).

The tissue culture fluids used in the SAIDS transmission experiments were assayed for antigens of MPMV, a type D retrovirus originally isolated from a rhesus monkey with a spontaneously occurring breast tumor (*7*). Viral pellets prepared by ultracentrifugation showed complete competition in a homologous radioimmunoassay (RIA) for the MPMV core antigen p27 (Fig. 3A). The slopes of the competition curves were similar to the slope of the MPMV standard indicating a close antigenic relationship to MPMV p27. The endogenous type D virus of langur monkeys (*Presbytis* species) (PO-1-Lu) also competed in the assay. This result was not surprising since PO-1-Lu is genetically related to MPMV (*7*). In contrast, pellets prepared from C1132 and C1055 did not compete in a homologous RIA for the MPMV envelope glycoprotein gp70 (Fig. 3B), even when viral antigen concentrates were 250-fold more than necessary for detectable competition. Mouse mammary tumor virus, Rauscher murine leukemia virus, and human T-cell leukemia virus type 1 (HTLV-1) did not compete in either assay. Thus the gp70 in the culture fluids may be antigenically different from MPMV gp70 or may have been sheared during ultracentrifugation. This

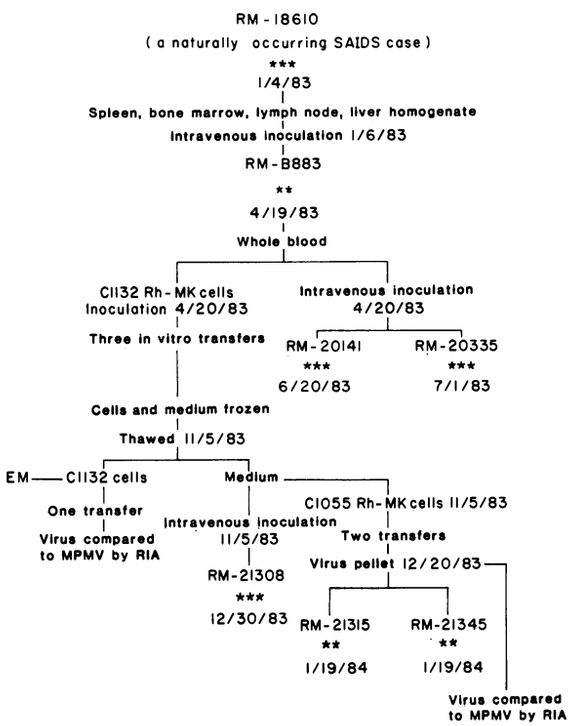

Fig. 1. Schematic diagram of the SAIDS transmission studies which led to the isolation of a type D retrovirus and production of disease. Symbols: **, alive with overt clinical SAIDS; ***, dead from SAIDS. Dates are shown for the different steps of the studies. Numbers prefixed by RM- are identification numbers for rhesus monkeys. The monkeys were colony-bred and maintained in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. RM-18610 was a 3-year-old female. The other monkeys are also female and range in age from 1 to 2 years. To initiate the culture C1132, we exposed confluent primary rhesus monkey kidney cells (Rh-MK; M.A. Bioproducts, Walkersville, Maryland) to whole blood for 18 hours, then washed the cells free of blood and maintained them with minimum essential medium (Gibco) containing 10 percent fetal calf serum, 100 m*M* glutamine, and 100 U of penicillin and 100 μg of streptomycin per milliliter. C1055 was initiated by treating subconfluent primary Rh-MK cells with Polybrene (5 μg/ml) for 24 hours and then inoculating the cultures with tissue culture fluid from C1132 (*EM*, electron microscopy).

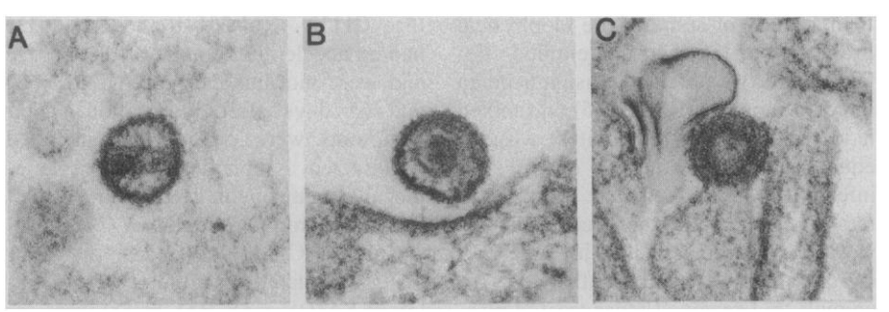

Fig. 2. (A and B) Transmission electron microscopy of thin sections of (A) C1132 cells and (B) C1281 cells stained with uranyl acetate and lead citrate. Cells were trypsinized, centrifuged, and fixed in Karnovsky's solution (*18*). (A) A virion is shown with the cylindrical core characteristic of type D retroviruses (*7*). (B) a similar particle is shown sectioned perpendicular to the virion in (A). (C) C1132 cells containing a budding virion with a ring-shaped core characteristic of type D retroviruses. (×100,000)

latter explanation seems unlikely since pelleted virus was highly infectious for rhesus monkeys and Rh-MK cells, and the infectious agent sedimented as an enveloped virus in a sucrose gradient.

Pellets prepared by ultracentrifugation of medium from C1055 were found to be negative in an RIA broadly reactive for type C viruses. In this assay we used antiserum (*8*) prepared in goats by sequential immunization with purified core proteins of several type C retroviruses. The radiolabeled antigen in the assay was the p28 core protein of the endogenous primate type C virus (CPC-1). The viral pellets were also negative when tested in an RIA for the HTLV-1 core antigen p24. These results suggest that type C viruses are not present in C1055.

Studies were also conducted at the National Institutes of Health. Pooled serum from two monkeys (RM-20414 and RM-20322) with spontaneously occurring SAIDS at the California Primate Research Center was sent to NIH where it was inoculated into two male rhesus monkeys, RM-B991 and RM-B992, both of which developed SAIDS. Pooled filtrates of serum (0.45 μm pore size) from these monkeys were inoculated into RM-E17. When RM-E17 developed advanced SAIDS, 1.0 ml of serum was collected and used to inoculate primary bone marrow cells (5×10^7 cells per 75-cm^2 vessel) obtained from a normal rhesus monkey. Cultures were maintained at 37°C in enriched McCoy 5a medium containing 0.1 μ*M* hydrocortisone and 25 percent fetal calf serum. After five serial passages, high reverse transcriptase activity was detected in fluids collected from adherent fibroblast cells of the bone marrow cell culture. After four additional passages of the infected fibroblasts, a virus was purified from the culture fluids by isopycnic banding in a neutral sucrose gradient. The fraction occurring in the ultraviolet absorption peak at 1.15 g/ml was inoculated into two rhesus monkeys (RM-E427 and RM-B959). Both animals developed SAIDS after 5 weeks and died at 8 weeks. Type D retrovirus was seen by electron microscopy in the fraction occurring at 1.15 g/ml, and the reverse transcriptase activity associated with this band showed a preference for Mg^{2+} when tested with polyriboadenylate · oligodeoxyribothymidylate$_{12 \text{ to } 18}$ and polyribocytidlyate · oligodeoxyriboguanylate$_{12 \text{ to } 18}$ (*9*).These are all characteristics of type D retroviruses.

Thus a type D retrovirus that is partially related to MPMV appears to be the etiologic agent of SAIDS in the macaques at the California Primate Research Center. This virus contains the core protein but not a detectable envelope glycoprotein of MPMV and has been repeatedly isolated from cells inoculated with blood from monkeys with SAIDS. Its etiologic role in SAIDS must be further confirmed by neutralization with appropriate antisera and with molecular and biologically cloned isolates. A similar virus grown in Raji cells has been obtained from macaques with SAIDS at the New England Regional Primate Center (*10*), although successful induction of SAIDS with virus grown in Raji cells was not reported. The restriction endonuclease cleavage pattern of our retrovirus isolate appears to be identical to that of the New England isolate (*10, 11*).

The association of this type of retrovirus with SAIDS is not unexpected. After the initial isolation of MPMV (*7*), early passage virus was inoculated into newborn rhesus monkeys and, although no tumors occurred, almost all animals developed a syndrome very similar to SAIDS (*12*). However, later in vitro passages of MPMV were nonpathogenic for newborn rhesus monkeys (*12*). Our review of the histopathology of one such monkey dying in 1972 showed lymphoid depletion typical of SAIDS. A mixture of type C and type D retroviruses was also recently isolated from a pigtailed macaque (*Macaca nemestrina*) with enzootic retroperitoneal fibrosis at the University of Washington Primate Center (*13*). This isolate was lost during cultivation in vitro and is not available for comparison with the SAIDS retrovirus (*13*).

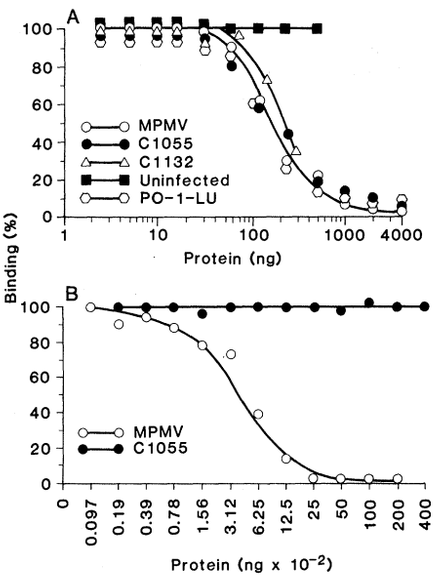

Fig. 3. Homologous RIA for (A) MPMV p27 and (B) MPMV gp70. Pellets obtained by ultracentrifugation of cultures were disrupted by 1 hour of incubation in 0.01*M* tris-HCl, *p*H 7.8, 0.1*M* NaCl, 0.001*M* EDTA, 0.1 percent Triton X-100 and 0.05 percent sodium desoxycholate, and then tested in twofold serial dilutions for ability to compete with ^{125}I-labeled viral proteins for binding limiting amounts of antisera. Antisera and unlabeled antigen were incubated for 1 hour at 37°C, and then ^{125}I-labeled antigen was added (20,000 count/min). After incubation for 1 hour at 37°C and overnight at 4°C, antigen-antibody complexes were precipitated by addition of 20 μl of *Staphylococcus aureus* (10 percent) and centrifugation at 2500*g* for 30 minutes. Radioactivity in the pellets was determined in a gamma counter. All the results were normalized to 100 percent binding in the absence of competing antigens.

These results focus further attention on the role of retroviruses in the etiology of human AIDS (*14*). They also indicate that the blood stream is a source of the SAIDS virus. However, in contrast to the murine (*15*) and feline (*16*) models of retrovirus-induced immunosuppression, the amount of virus in SAIDS plasma and tissue is very low ($\leq 10^4$ particles per milliliter), (*17*) and the virus is a type D rather than type C retrovirus. This simian model should prove useful for determining how an infectious retrovirus causes depletion of lymphoid cells and for the development of measures for controlling SAIDS in primates.

PRESTON A. MARX*
DONALD H. MAUL
KENT G. OSBORN
NICHOLAS W. LERCHE
PEGGY MOODY
LINDA J. LOWENSTINE
ROY V. HENRICKSON
California Primate Research Center, University of California, Davis 95616
LARRY O. ARTHUR
RAYMOND V. GILDEN
Frederick Cancer Research Facility, Frederick, Maryland 21701
MANETH GRAVELL
WILLIAM T. LONDON
JOHN L. SEVER
Infectious Diseases Branch, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20205
JAY A. LEVY
Cancer Research Institute, University of California Medical Center, San Francisco 94143
ROBERT J. MUNN
MURRAY B. GARDNER
Department of Pathology, School of Medicine, University of California, Davis 95616

References and Notes

1. R. V. Henrickson *et al.*, *Lancet* **1983-I**, 388 (1983).
2. R. D. Hunt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5085 (1983); N. L. Letvin *et al.*, *Lancet* **1983-II**, 599 (1983).
3. M. Gravell *et al.*, *Science* **223**, 74 (1984).
4. K. G. Osborn *et al.*, *Am. J. Pathol.* **114**, 94 (1984).
5. W. T. London *et al.*, *Lancet* **1983-II**, 869 (1983).
6. M. A. Gonda *et al.*, *Arch. Virol.* **56**, 297 (1978).

7. D. Fine and G. Schochetman, *Cancer Res.* **38**, 3123 (1978).
8. R. V. Gilden, *Adv. Cancer Res.* **22**, 157 (1975).
9. M. Gravell, in preparation.
10. M. D. Daniel *et al.*, *Science* **223**, 602 (1984).
11. E. Hunter, personal communication.
12. D. L. Fine *et al.*, *J. Natl. Cancer Inst.* **54**, 651 (1975); D. Fine, personal communication.
13. E. Hefti, *Virology* **127**, 309 (1983); S. Panem, personal communication.
14. M. Essex *et al.*, *Science* **220**, 859 (1983); E. P. Gelmann *et al.*, *ibid.*, p. 862; R. C. Gallo *et al.*, *ibid.*, p. 865; F. Barré-Sinoussi *et al.*, *ibid.*, p. 868.
15. H. Friedman and W. S. Ceglowski, in *Progress in Immunology*, B. Amos, Ed. (Academic Press, New York, 1971), pp. 815–829.
16. M. Essex, *Am. J. Vet. Res.* **34**, 809 (1973).
17. P. Marx, in preparation.
18. M. J. Karnovsky, *J. Cell Biol.* **27**, 137A (1965).
19. We thank M. Bryant, R. Cardiff, D. Fine, E. Hunter, and C. Barker for discussions; R. Cork, J. Katilus, B. Bencken, J. Bess, A. Spinner, R. Beards, J. Tribble, J. Lund, and M. Bleviss for technical assistance; L. Click and A. Dean for manuscript preparation; C. Sarason for artwork; and the Federal Express Corporation for overnight delivery. Supported by grants RR00169, AI20573-01, contract N01-CO-23910, and Interagency Agreement from Blood Division, NHLBI with Division of Research Resources, NIH, and by special appropriation from the state of California.
* To whom reprint requests should be addressed.

20 January 1984; accepted 13 February 1984

T-Cell Growth Factor Gene: Lack of Expression in Human T-Cell Leukemia-Lymphoma Virus-Infected Cells

Abstract. *Activated mature T cells require T-cell growth factor (TCGF) for continuous proliferation. However, many mature T cells infected with human T-cell leukemia-lymphoma virus grow independently of exogenously added TCGF. It is now reported that cells infected with this virus also lack detectable TCGF messenger RNA (less than one copy per cell) and thus do not produce their own growth factor. The results apparently rule out an autostimulation mechanism of growth control.*

T-cell growth factor (TCGF, interleukin 2) is required for the continuous proliferation of specifically activated mature T lymphocytes (*1*). Similarly, some neoplastic mature T cells require TCGF for their growth in vitro, although in some cases without the requirement of prior antigen and lectin activation (*2*). This is apparently because at least some of these cells already possess TCGF receptors (*3*). Some mature T cells infected with human T-cell leukemia-lymphoma virus (HTLV) require TCGF for growth early in culture. Subsequently, many of the HTLV-infected cell lines become independent of exogenously added TCGF (*4*). Some of these cell lines constitutively produce and respond to their own TCGF (*3*), while other cell lines do not elaborate detectable extracellular TCGF (*5*). The question thus arises whether the latter cell lines are truly independent of TCGF or produce small quantities of TCGF, sufficient for growth but undetectable by conventional assay procedures. Moreover, it is possible that these HTLV-infected cells produce TCGF that is not externalized and that would not be detected in extracellular medium. A more direct answer to this question could be obtained from a study of TCGF messenger RNA (mRNA) in these cells. This is now possible because a human TCGF complementary DNA (cDNA) sequence has been molecularly cloned (*6*, *7*), providing a sensitive probe for evaluating the expression of the TCGF gene. We have previously demonstrated that the production of TCGF is regulated at the transcriptional level (*7*). Therefore, if these cell lines synthesize TCGF, whether externalized or not, they would be expected to contain TCGF mRNA. Here we show that several of the TCGF-independent cell lines do not contain detectable TCGF mRNA, suggesting that the immortalization of mature T cells by HTLV may sometimes be by mechanisms that bypass the TCGF-TCGF receptor system.

The TCGF gene recently cloned from a normal human lymphocyte cDNA library (*7*) was identical to the clone from the human lymphoma Jurkat cell line (*6*) and contained the complete coding sequence for TCGF, in addition to 5′ and 3′ untranslated sequences. We have shown that this gene is transcribed into an 11*S* to 12*S* mRNA in all human TCGF-producing cells (*7*). To determine if such an mRNA species was also present in HTLV-infected cells, we analyzed polyadenylate [poly(A)]-containing RNA from these cells by the Northern blot procedure, using cloned TCGF DNA as a probe (Fig. 1). The cell lines examined included those derived from HTLV-positive T-cell malignancies (HUT 102, MO, MI, and MJ), those obtained by HTLV infection of normal human cord blood and bone marrow cells in vitro (C5/MJ, C10/MJ, B2/UK, and MT-2), and an uninfected mature neoplastic T-cell line (HUT 78) (see legend to Fig. 1). The Jurkat cell line, which produces abundant TCGF upon stimulation with phytohemagglutinin (PHA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (*7*, *8*), served as a positive control.

As expected, 11*S* to 12*S* TCGF mRNA was readily detected in stimulated Jurkat cells. A similar mRNA species was also detected in HTLV-infected HUT 102 and MO cells, and also in HUT 78 cells. We estimate (*9*) that Jurkat, MO, and HUT 102 cells, respectively, contain about 40 to 50, 5 to 10, and less than 5 copies of TCGF mRNA per cell. None of the other HTLV-infected cells contained detectable TCGF mRNA. Since TPA in combination with PHA increases the level of TCGF mRNA in some cells (*7*), a number of HTLV-infected cells were also examined after treatment with TPA and PHA. This treatment did not cause

Fig. 1. Expression of TCGF gene in HTLV-infected cells. Poly(A)-selected RNA, size-separated by agarose gel electrophoresis, was hybridized with labeled cloned TCGF DNA. Lanes 1 to 14 are for RNA from: 1, PHA plus TPA-stimulated Jurkat cells; 2, TCGF-independent HUT 78 cells; 3, TCGF-independent HUT 102 cells; 4, another preparation of TCGF-independent HUT 102 cells; 5, TCGF-independent C5/MJ cells; 6, TCGF-dependent C5/MJ cells; 7, TCGF-independent C10/MJ cells; 8, TCGF-independent C10/MJ cells treated with TPA-PHA; 9, TCGF-independent B2/UK cells; 10, TCGF-independent B2/UK cells treated with TPA-PHA; 11, TCGF-independent MT-2 cells; 12, TCGF-dependent MI cells; 13, TCGF-independent MO cells; and 14, TCGF-independent Molt 4 cells (immature T). Poly(A)-selected RNA was obtained as described (*15*). After denaturation at 65°C in 50 percent formamide, RNA (10 μg per lane) was subjected to electrophoresis in 1 percent agarose slab gel containing 6 percent formaldehyde and transferred to a Zeta Probe membrane (Bio-Rad Laboratories) by electroelution (*7*). Hybridization with ^{32}P-labeled nick-translated cloned TCGF DNA was performed at 37°C for 16 hours in a mixture containing 50 percent formamide, five times standard sodium chloride and sodium citrate (SSC, 0.15*M* NaCl and 0.015*M* sodium citrate, *p*H 7), 0.05*M* sodium phosphate buffer (*p*H 7), five times PM (0.02 percent each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll 400), yeast RNA (200 μg/ml), denatured DNA (20 μg/ml), 0.1 percent sodium dodecyl sulfate (SDS), and 10 percent dextran sulfate. The membrane was subsequently washed repeatedly with SSC and 0.1 percent SDS at 65°C, air dried, and exposed to a Kodak XAR film with the use of intensifying screens. Where indicated, cells were treated with TPA (10 ng/ml) and PHA (1 μg/ml) for 20 hours.