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Productive, Persistent Infection of Human Colorectal Cell Lines with Human Immunodeficiency Virus

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Thirteen adherent human non-lymphocyte cell lines were tested for their susceptibility to infection by human immunodeficiency virus. Productive infection could be demonstrated in three of five colorectal carcinoma cell lines examined; the other eight human non-lymphocyte cell lines were uninfectible. A susceptible colon carcinoma cell line (HT29), as well as normal colonic mucosa, was shown to contain a 3.0-kilobase species of poly(A)$^+$ CD4 RNA, whereas uninfectible colon carcinoma and rhabdomyosarcoma cell lines synthesized no detectable T4 RNA. A persistently infected colon carcinoma cell line was established that continued to produce progeny human immunodeficiency virus for more than 10 weeks postinfection.

The human immunodeficiency virus (HIV) has been isolated from the peripheral blood (14, 23), semen (11, 23, 26), vaginal fluid (24, 25), saliva (11, 23), and tears (6) of exposed individuals. HIV is not a casually transmitted agent; among high-risk groups the virus is spread sexually or subsequent to parental inoculation attending the use of illicit drugs or administration of blood products (3).

The clinical manifestations of HIV infection are thought to be due to selective tropism for the OKT4/Leu-3 subset of human T lymphocytes both in vivo (9, 10) and in vitro (4, 15). The virus readily infects and kills lymphocytes expressing the CD4 molecule. Several reports have also shown that HIV is capable of infecting certain established human B cell lines (17, 20) and monocyte-macrophage cell lines (7, 13, 17). In vivo, it has also been noted that macrophages are the major targets for HIV in the central nervous system (16).

We have recently evaluated the susceptibility of several human and nonhuman cell lines to transfection with an infectious molecular clone of HIV (1). Those studies demonstrated that viral RNA and proteins were synthesized and assembled into infectious progeny virions in a variety of nonlymphoid human, monkey, mink, and mouse cells after the introduction of cloned proviral DNA. Expression of HIV was monitored by in situ hybridization or cocultivation of transfected cells with a CD4$^+$ human T cell line. The results obtained suggested the absence of any intracellular obstacle to viral RNA or protein synthesis and assembly and indicated that interaction of HIV particles and their receptor(s) during the initial stages of virus infection was the major determinant of cell tropism.

During the course of the transfection studies, experiments were initiated to ascertain whether some of the same human lines could be directly infected with virus. The 14 cell lines listed in Table 1 were infected with the lymphadenopathy-associated virus (LAV) strain (2) of HIV at a multiplicity of infection (MOI) of approximately 0.1. A3.01 cells, a CD4$^+$, IL-2-independent, and hypoxanthine-aminopterin-thymidine-sensitive derivative of the CEM cell line, had previously been shown to support productive virus infection efficiently (5) and served as a positive control. Since none of the cell lines listed in Table 1, except A3.01, would be expected to express the putative receptor for HIV, it seemed very unlikely that progeny virions generated from a primary infection could spread to other cells in the culture. Virus infection was initially evaluated by in situ hybridization 3, 7, and 14 days postinfection. Only 2 of the 11 non-lymphocyte cell lines (the HT29 and SW480 lines, both of which were derived from colon carcinomas) synthesized HIV RNA in response to viral infection (Fig. 1A and B; Table 1). Approximately 10^6 cells were analyzed in each experiment. Those cells containing ≥10 grains above background (0 to 5 grains per cell) were scored as positive. The percentages of HT29 cells expressing viral RNA were 0.05, 0.1, and 0.05 on days 3, 7, and 14 postinfection, respectively. The SW480 colon carcinoma line was less susceptible to viral infection, containing 0.01% positive cells on day 7. Infection of HT29 and

TABLE 1. Susceptibility of human cell lines to HIV infection[a]

Cell line	Description of cells	Infection monitored by:	
		In situ hybridization	Cocultivation
A3.01	T-cell leukemia	+	+
A204	Rhabdomyosarcoma	−	−
CHP126	Neuroblastoma	ND[b]	−
SVG	Fetal glial	−	ND
HMB2	Melanoma	−	−
A549	Lung carcinoma	−	−
CAPAN-1	Pancreatic carcinoma	−	−
SK-OV-3	Ovarian carcinoma	−	−
T47D	Breast carcinoma	−	−
CaCO-2	Colon carcinoma	−	−
SK-CO-1	Colon carcinoma	−	−
HT-29	Colon carcinoma	+	+
SW480	Colon carcinoma	+	+
SW1463	Rectal carcinoma	ND	+

[a] The titer of the LAV stock used in these experiments was determined by end point dilution and RT assay in A3.01 cells (5). This stock contained approximately 10^6 infectious units/ml. Adherent cell cultures were incubated with virus (MOI, 0.1) in the presence of Polybrene (2 μg/ml) for 16 h, at which time fresh medium was added.

[b] ND, Not done.

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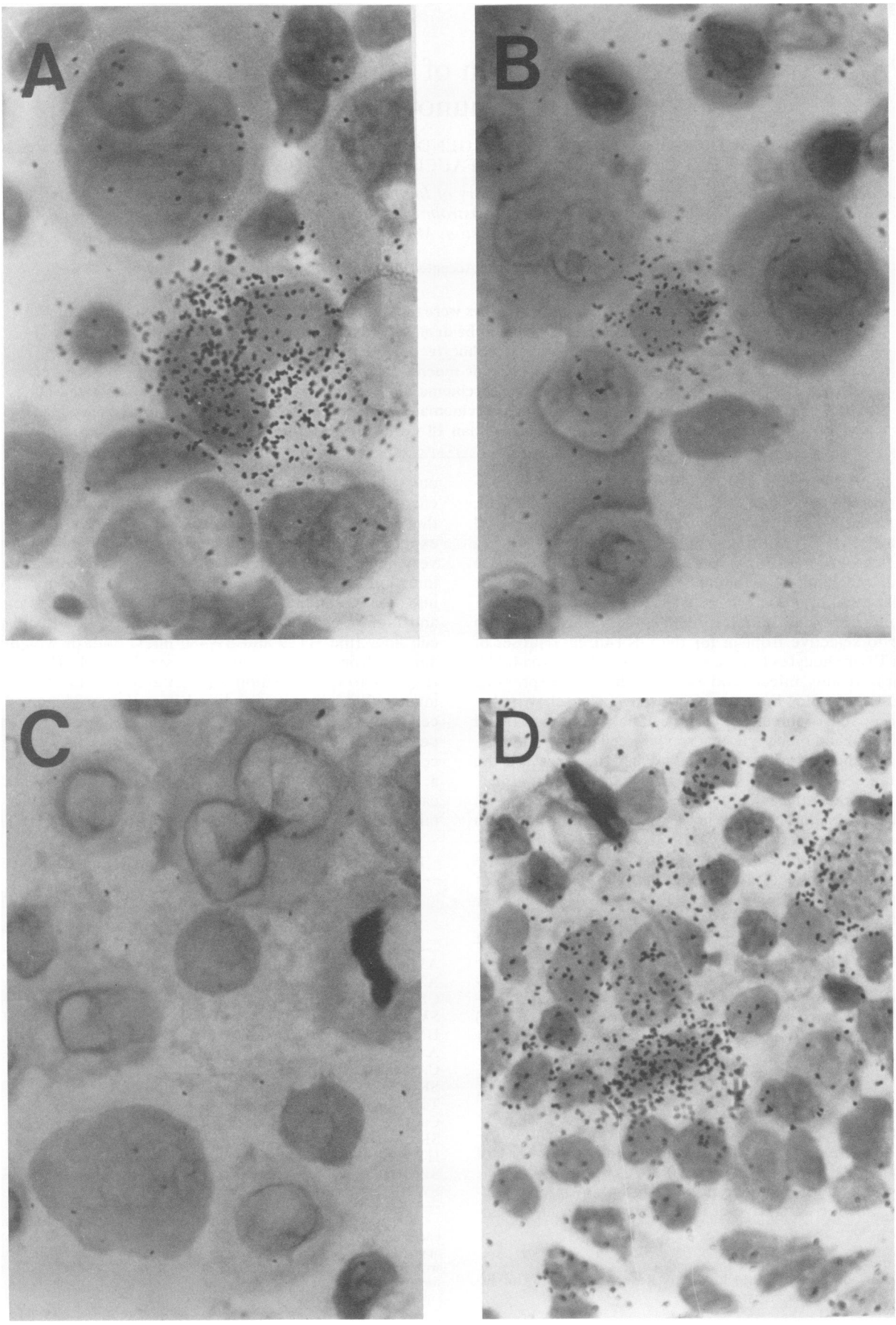
A
B
C
D

SW480 cells with LAV was repeated, and the same in situ hybridization results were obtained. The amount of HIV RNA synthesized in positive HT29 and SW480 cells, as monitored by the number of grains, was comparable to that present in positive cells from a virally infected $T4^+$ clonal cell line (Fig. 1D) obtained by preparative flow cytometry and limiting dilution of $OKT4^+$ peripheral-blood lymphocytes from a normal individual.

As a control for this experiment, RNA probes with the same polarity as viral mRNA were synthesized and used in in situ hybridization experiments. No specific annealing was observed in duplicate experiments with any of the 12 cell lines listed in Table 1. In addition, no hybridization was observed when RNA probes complementary to HIV mRNA were annealed to preparations of uninfected cell cultures (Fig. 1C).

The expected absence of the T4 determinant on the surface of the non-lymphocyte cells listed in Table 1 would most likely preclude the secondary spread of input HIV. To amplify any progeny virions produced, $CD4^+$ A3.01 cells were cocultivated with the infected non-lymphocyte cultures 21 days postinfection. Virus was detected in the HT29 and SW480 colon carcinoma cell lines cocultured with A3.01 cells (Fig. 2) by reverse transcriptase (RT) assays. In addition, production of HIV could also be demonstrated in a third colorectal carcinoma line, SW1463, by the cocultivation procedure; none of the other nine non-lymphocyte lines examined were susceptible to virus infection (Table 1). Progeny virus particles appeared much earlier in HT29 cells than in the SW480 and SW1463 cell lines (Fig. 2). This most likely reflects a more efficient primary viral infection of HT29 cells and is consistent with the higher proportion (0.1%) of positive cells monitored by in situ hybridization.

In experiments using the cocultivation-RT assay procedure to detect progeny virions, we were initially concerned about the possibility that the input virus inoculum might adsorb to or associate with the nonlymphoid cell cultures for long periods of time yet fail to initiate a productive infection. Any residual replication-competent virus present in the system would be capable of infecting the A3.01 cells which were added to amplify the HIV. This would register as a false-positive result. This is actually what occurred in a preliminary experiment in which $CD4^+$ A3.01 cells were cocultivated with non-lymphocyte cultures early in infection. The "progeny" virus detected by the coculture-RT assay procedure in some of the infected cell lines 7 days postinfection clearly reflected the presence of residual input virus since simultaneous in situ hybridization analyses of the same cells were invariably negative. In contrast, when the A3.01 cells were added 21 days postinfection, progeny virus could only be demonstrated in the three colorectal carcinoma cell lines; HIV was never detected in the nine other non-lymphocyte lines examined under these conditions (viz., addition of A3.01 cells 21 days postinfection and maintenance of the cocultures for 24 days).

An intriguing aspect of HIV infection of colorectal carcinoma cell lines that also validated the results obtained with the cocultivation-RT assay procedure was the apparent chronicity of virus production. A persistently infected HT29 cell culture was established that continued to synthesize

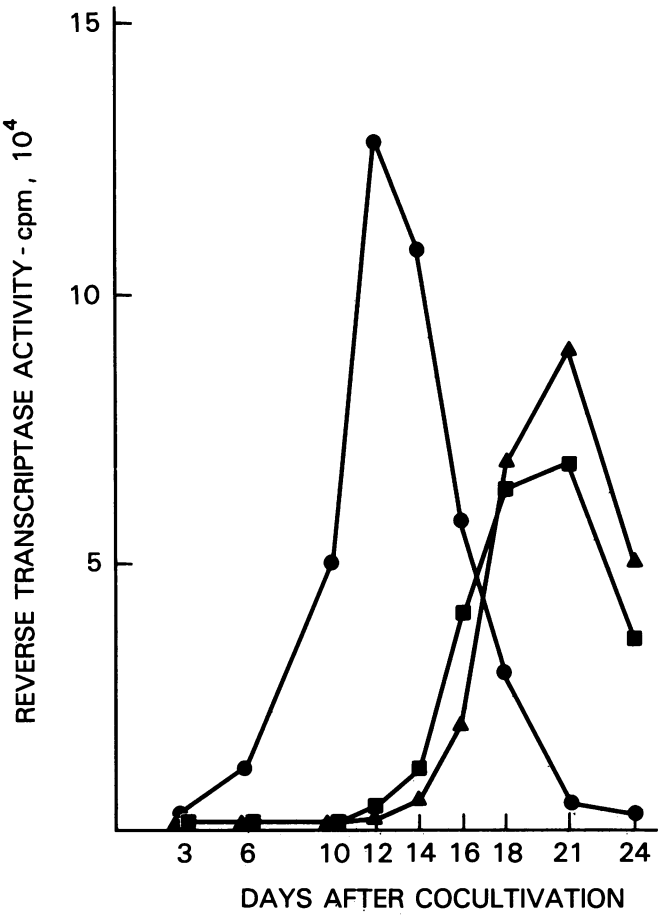

FIG. 2. Detection of progeny HIV produced in three human colorectal carcinoma cell lines. HT29 (●), SW480 (▲), and SW1463 (■) cells were infected with LAV at an MOI of 0.1. Twenty-one days postinfection, 2×10^6 cells were cocultured with 10^6 A3.01 cells, and RT activity in the culture medium (65 μl) was monitored (5) on the days indicated.

progeny virions more than 10 weeks postinfection (Table 2). At the present time, the state of the viral genome, as well as the mechanism(s) responsible for continuous virus production, is unknown and is being actively investigated. It is not clear whether HIV is chronically produced by a small fraction of HT29 cells or whether a minority of the cells are susceptible to infection, are killed during the process of viral replication, and release progeny particles which could then infect other susceptible colorectal carcinoma cells.

The colorectal carcinoma cell lines used in this study were characterized in our laboratory subsequent to their receipt from the American Type Culture Collection (Rockville, Md.). By using a monoclonal antibody (COL-4) which cross-reacts with the carcinoembryonic antigen present in human colon carcinomas (21), we observed reactivity with the five colorectal carcinomas, the CAPAN-1 pancreatic carcinoma, and the SK-OV-3 ovarian carcinoma lines listed in Table 1 by using a modification of the avidin-biotin-immunoperoxidase technique (8).

FIG. 1. In situ hybridization of HIV-infected cells. The SW480 (A) and HT29 (B) colon carcinoma cell lines were infected with the LAV strain (2) of HIV at an MOI of 0.1; a cloned human $T4^+$ cell line propagated in the presence of phytohemagglutinin (D) was infected at an MOI of 0.01. Cells were harvested 7 (SW480 and HT29) or 9 (infected $T4^+$ lymphocyte line) days postinfection and subjected to in situ hybridization as previously described (1). Mock-infected HT29 (C) cells were similarly examined. Magnification, ×400.

TABLE 2. Persistence of HIV infection in HT29 cells[a]

Day cocultivation was initiated	Day post coculture	RT activity (10^3 cpm/65 μl)
21	3	3.5
	6	10.5
	10	50.0
	12	130.0
	14	110.0
	16	60.0
	18	35.0
	21	4.5
35	3	2.1
	6	9.8
	10	47.0
	12	115.0
	16	45.0
	18	17.0
45	3	3.5
	7	4.3
	10	3.7
	12	12.0
	14	100.0
	16	87.0
70	3	4.0
	7	3.5
	10	86.0
	12	108.7
	14	54.5

[a] Infected HT29 cells (2×10^6) were cocultivated with A3.01 cells (10^6) on the days indicated, and the kinetics of RT activity in the medium of the coculture were determined.

The colorectal carcinoma cell lines were also examined for expression of CD4 gene products which have been shown to be associated with the cellular receptor for HIV (4, 15, 19). Despite numerous attempts, we were unable to detect T4 proteins unambiguously on the surface of susceptible human colon carcinoma cell lines by using monoclonal antibodies in indirect immunofluorescence, flow cytometry, or immunoperoxidase assays. In contrast, CD4-specific RNA could be demonstrated in infectible cell lines but not in those refractory to virus infection (Fig. 3). Poly(A)$^+$ RNA was prepared from HT29 (colon carcinoma), CaCO2 (colon carcinoma), A3.01 (T4$^+$ lymphocytic leukemia), and A204 (rhabdomyosarcoma) cell lines and analyzed by Northern blot hybridization with a T4 cDNA probe (18). A strongly reactive 3.0-kilobase RNA species was readily demonstrable in the T4$^+$ A3.01 lymphocyte line (Fig. 3, lanes a and b). A comigrating species of CD4 RNA was also present in the infectible HT29 colon carcinoma cell line (Fig. 3, lane c) but not in the CaCO2 colon carcinoma or A204 rhabdomyosarcoma cell line (Fig. 3, lanes d and e), both of which were refractory to HIV infection (Table 1). Based on band intensity and exposure time, we estimate the amount of stable CD4 RNA in HT29 cells to be approximately 2% of that present in the A3.01 lymphocytic leukemia line. In a separate experiment, poly(A)$^+$ RNA was prepared from human sigmoid colon mucosa obtained from a patient undergoing resection for diverticulosis. The mucosa was dissected away from the underlying muscularis and submucosa layers. Gross and histological examinations revealed no evidence of inflammation or perforation. The 3.0-kilobase CD4-reactive band was readily visualized in the colon mucosal RNA preparation (Fig. 3, lane f).

The demonstrated susceptibility of three of five human colorectal carcinoma cell lines to infection by HIV has potential epidemiologic implications. A series of experiments are currently under way. Colorectal biopsy specimens from homosexual acquired immunodeficiency syndrome patients are being examined by in situ hybridization. Attempts are also being made to infect primary colon cells in culture to establish a more physiologic in vitro cell culture system.

Although only a small fraction (up to 0.1%) of HT29, SW480, and SW1463 cells were shown to be directly infectible with HIV, this level may be significant if considered in the context of infection in vivo. When peripheral-blood lymphocytes obtained from individuals with acquired immunodeficiency syndrome-related complex or acquired immunodeficiency syndrome were evaluated by in situ hybridization, only 5 to 10 per 10^6 cells actively synthesized viral RNA (12; S. Koenig and H. Gendelman, unpublished data). In a related experiment, we examined the effects of phytohemagglutinin on the susceptibility of a T4 clonal line (obtained by preparative flow cytometry of normal peripheral blood lymphocytes followed by limiting dilution) to infection with HIV. In the absence of phytohemagglutinin, only 0.01 to 0.1% of infected T4 cells were positive by in situ hybridization during a 30-day observation period (S. Koenig, unpublished data). In contrast, more than 50% of the T4 cells actively synthesized viral RNA if they had been stimulated

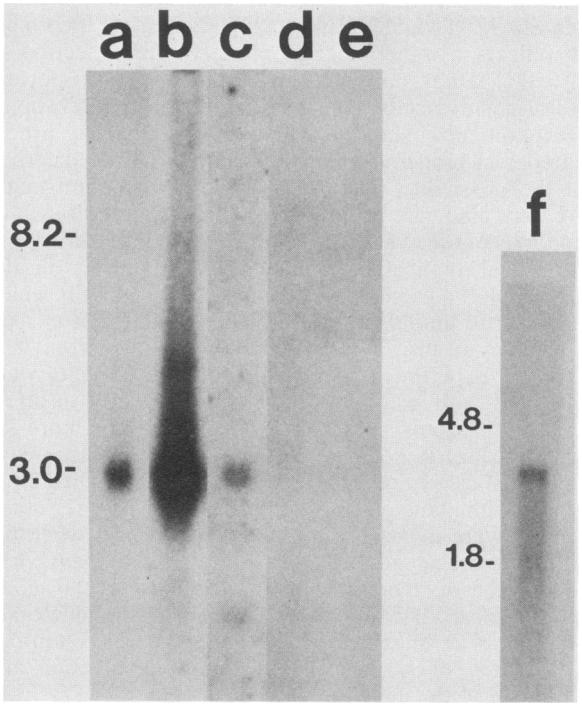

FIG. 3. T4 RNA expression in different human cells. Poly(A)$^+$ RNA (5 μg) was electrophoresed through a 1% agarose–formaldehyde gel, blotted onto nitrocellulose, hybridized with a ^{32}P-labeled T4 cDNA probe, and washed as previously described (22). RNAs were prepared from (lanes) T4$^+$ A3.01 lymphocytic leukemia cells (a and b), HT29 colon carcinoma cells (c), CaCO2 colon carcinoma cells (d), A204 rhabdomyosarcoma cells (e), and normal human colon mucosal cells (f). Autoradiogram exposures were for (lanes) 5 (a), 73 (b and c), 96 (d and e), and 48 (f) h. Size markers were 8.2- and 3.0-kilobase murine leukemia and mink cell focus-forming viral RNAs (lanes a to e) and 28S and 18S rRNAs (lane f).

with phytohemagglutinin before infection. The small fraction of susceptible colorectal carcinoma cells we observed could reflect the low levels of CD4 expression detected in these cells. Since similar amounts of T4 RNA were also demonstrated in normal colon mucosa, it is tempting to hypothesize the establishment of a chronic infection at this site in vivo which could ultimately result in dissemination of the virus to cells of the lymphocytic and monocytic series and, in some cases, lead to the development of the acquired immunodeficiency syndrome disease complex.

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