The Search for Infectious Viral DNA in Human Leukemic Cells

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That human leukemia has a viral origin has been suggested many times (see [6] for references). Leukemic cells have been shown to bear intracellular virus-like particles [1,4], to share nucleotide sequences with murine [1] or primate [4] viruses, and to synthesize proteins that cross-react with antibodies to virus-specific proteins [18]. Some authors reported the release of C-type particles from leukemic cells subjected to in vitro culture conditions [11,12,13,16], and Gallagher and Gallo [3] have shown budding C-type virus in leukemic cells sustained proliferating in vitro with medium conditioned by a particular human embryonic cell culture. This virus, now called HL23V, was further studied and found to be infectious for a wide variety of cells. Viruses growing in these new hosts were referred to as "secondary viruses" [20].

During horizontal transmission animal C-type viruses are known to synthesize a provirus composed of double-stranded DNA of about 6×10^6 daltons which, because of its infectivity, can be conveniently detected in a transfection assay (see [9] for review). Viral genetic information, endogenously carried in the cellular chromosome, is usually not infectious ([2], for exceptions see [17]). In human leukemia an infectious provirus should be found if the disease arises from a horizontal spread of a leukemia virus. We report here on our search for such a provirus.

In initial experiments [7] the DNA was extracted from secondary HL23V-1 virus-infected bat B88 cells, and used to transfect both human rhabdomyosarcoma A204 and bat B88 cell cultures. Transfection assays were carried out using calcium phosphate [5] or DEAE-dextran [8] techniques. In these assays 20 to 50 μg DNA was delivered per about 10^7 recipient cells. The cells were then kept growing for about 1 month and assayed for reverse transcriptase activity in the culture medium. Four out of ten B88 and none out of five A204 cultures were found to produce C-type virus. Reverse transcriptase neutralization tests performed by Robert E. Gallagher have shown that viruses recovered in these transfections resemble simian sarcoma virus and baboon endogenous virus to approximately the same extent as does the parent HL23V-1 virus. We concluded that after horizontal transmission, the HL23V isolate synthesizes an infectious provirus. This was later confirmed by others [14].

In order to detect such a provirus before the rounds of virus replication in vitro, the DNA was extracted from fresh-frozen uncultured leukemic leukocytes from the HL-23 patient, and also from patients HL-7 with chronic myelogenous leukemia and HL-11 with chronic monomyelocytic leukemia.

Wong-Staal et al. [21] showed by molecular hybridization that leukemic tissues in these patients, unlike normal human tissues, contain baboon endogenous virus-specific DNA sequences suggesting that horizontal transmission of a primate virus had occurred among humans.

The results of transfection assays performed with these DNAs are shown in Table 1. It is clear that no infectious virus could be isolated. Some DNA-treated cultures, however, exhibited a transient burst of DNA polymerase activity in the culture medium. This is demonstrated in Fig. 1. To reproduce the burst, about 107 days after the first DNA treatment the cultures N° 38 and 39 and N° 44 and 45 were treated again with HL-7 and HL-11 DNA, respectively. Two control cultures received normal human foreskin DNA. This time the DNA treatment was repeated 17 times during 20 cell passages and 20 μg DNA was delivered per culture of 10^7 cells at each treatment. Reverse transcriptase activity was assayed weekly as in Fig. 1 and found to be within the background limits.

In further experiments HL-23 DNA (20 μg/10^7 cells) was assayed on feline embryo cells and also in a mixture of these cells and rabbit cornea cells. Transfection assay was carried out using the calcium phosphate coprecipitation technique, combined with the boost of DMSO [19] 4 h or 4 h and 10 days after transfection. Reverse transcriptase assays were carried out in 10 ml of the culture medium weekly for up to 2 months and provided only background counts.

The burst of DNA polymerase activity after transfection is reminiscent of the transient virus replication observed with HL23V-1 virus in various animal and human cells [20]. The burst shown in Fig. 1 may be due to the release of C-type particles. It is not known if these particles were specified by the transfecting DNA. We suspect that leukemic cells carry a defective provirus

Table 1. Transfection assays using DNA from fresh-frozen, uncultured human leukemic leukocytes

Experiment N°	Culture N°	Source of DNA (patient)	Recipient cells	μg DNA per culture	Reverse transcriptase assay	Number of passages after transfection
I	31–35	HL-7	B88, bat lung	50	negative	7
IIa	36–40	HL-7	SIRC, rabbit cornea	40	burst in N° 39	17
	41–45	HL-11	SIRC, rabbit cornea	40	burst in N° 45	17
	46–50	HL-23	SIRC, rabbit cornea	18	burst in N° 47	17
III	53–55	HL-23	A7573, dog thymus	20	negative	16
	56–58	HL-23	FEF-1, feline embryo	20	negative	lost at passage 5
IV	59–63	HL-23	FEF-1, feline embryo	40	negative	25

DNA was extracted from cells according to a modified Marmur's procedure ([8, 15], and unpublished data) and then administered to about 10^7 recipient cells using the Ca phosphate transfection technique [5]. The cells were passaged once weekly. Before each passage 10 ml of the culture medium were withdrawn, clarified at 10,400 g for 30 min, and the particulate fraction spun down in a Spinco Ti50 rotor at 38,000 rpm and 4°C for 60 min. The pellet was assayed for reverse transcriptase activity as described [10].

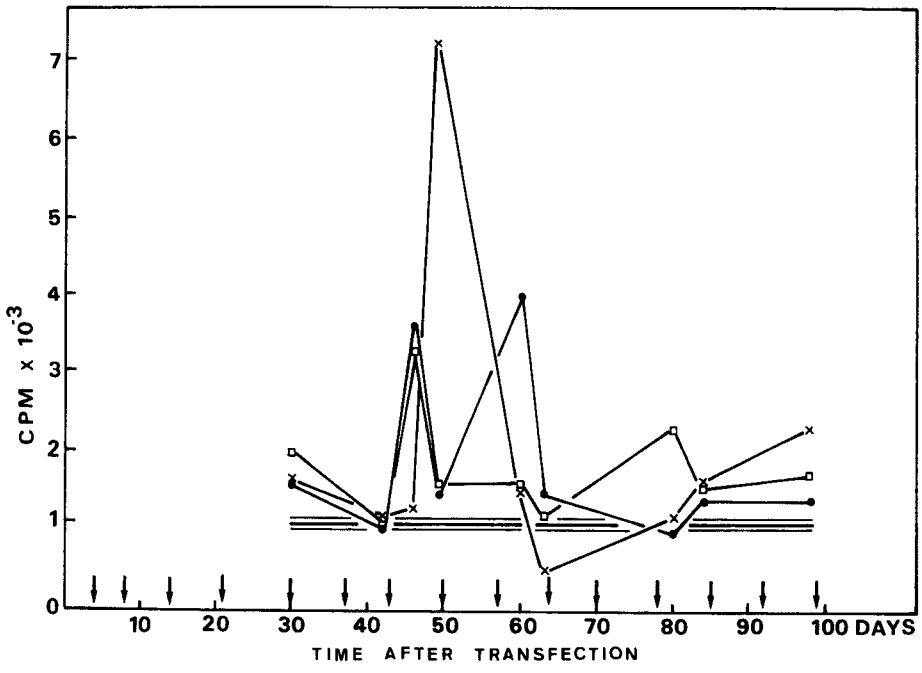

Fig. 1. Reverse transcriptase assay (given in cpm of incorporated ^{3}H-TMP) of the culture medium of DNA-treated cultures N° 39 (×), 45 (□), and 47 (●) at different times after transfection. Mean background counts and the standard error of the mean are given by a thick and two thin straight lines, respectively. Cell passages are indicated by arrows. Other conditions are described in Table 1

which gives rise to a poorly replicating virus in transfected cells. In this context the results of the last experiments would show that different procedures such as repeated transfections, co-culture with different animal cells, or DMSO boosts are all unable to render this virus fully infectious.

Other possibilities could not be eliminated. For instance, (i) no virus is recovered after transfection if the DNA used contains too small an amount of infectious provirus. In infections initiated by avian sarcoma virus in virus-resistant chicken cells no infectious provirus is detected when the virus replicates in less than one per 10^4 cells. (ii) The fact that endogenous proviruses are usually noninfectious [2] suggests that genetic elements of the endogenous provirus are located on different chromosomes or separated by spacers large enough to render the provirus inefficient in transfection. If this is also true of the human leukemia provirus, C-type particles may be produced after splicing in leukemic cells, but not after transfection of leukemic cell DNA.

In conclusion, transfection assays so far performed failed to detect infectious provirus in human leukemic cells. A possibility remains, however, that these cells carry a defective provirus or a provirus composed of more than one genetic element unable to generate, upon transfection, an infectious virus.

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