Murine and Simian C-Type Viruses: Sequences Detected in the RNA of Human Leukemic Cells by the c-DNA Probes

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Introduction

The detection of viral-related RNA sequences in human leukemic, human breast cancer, and other human cancer cells has been facilitated in recent years by molecular hybridization techniques of human ribonucleic acid to the complementary DNA (c-DNA) synthesized in-vitro by animal type – cRNA tumor viruses (1–5). Our laboratory has been interested in the search for such sequences in the nucleic acids of various types of acute and chronic human leukemias. The approach was based essentially upon the evaluation of the annealing rate of single stranded c-DNA to cellular RNA and DNA.

We report some of the annealing experiments we performed in the search for virus-like sequences in the nucleic acids of human acute and chronic leukemias with the aid of two synthetic c-DNA probes synthesized with the murine sarcoma-leukemia viruses (Moloney Isolate) (M-MSV-MLV) produced continously by the transformed rat cell line 78 A-1 and a simian probe synthesized with the simian (Wooly Monkey) sarcoma and simian sarcoma-associated viruses (SSV) produced by the Normal Rat Kidney NRK cell line.

Methods and Results

Both c-DNA probes were synthesized and prepared as described previously and represented extensive complementary copies of their respective 70S RNA genomes (6). However, the SSV probe seemed less uniform in terms of complementarity to its 70S viral RNA genome in that a 8 to 10-fold excesss of c-DNA was necessary to render the 70S RNA resistant to RNAase digestion in high salt (as compared to 2–3 fold excess in the case of M-MSV MLV).

Annealing reactions between the tritium labelled c-DNA of both viruses and the RNA of leukemic cells were performed as described previously (7) (or at higher temperatures : 68° in 4 x SSC). The rate of annealing was estimated by the S_1 nuclease assay (7). The percentage of hybridization was expressed by normalizing against the values given by the RNA of the respective virus producing cells (78 A_1 or NRK) taken as 100 percent, and after substraction of the background obtained by the blanks of c-DNAs processed without RNA.

Hybridization reactions with RNA from human leukemic and "normal" cells.

In previous experiments it was shown that hybrids of M-MSV(MLV)-c-DNA and human RNA were found in 22 out of 46 leukemias whereas none of the 10 controls tested (including material obtained from bone marrow cells, buffy coats and continuous human cell lines) was positive (8).

We report here the results obtained in 12 leukemias and 4 controls which were available for concomitent study with both the simian and the murine probes. Table I summarizes the results of this type of study. It can be seen that there is

Table I: Hybridization to human cellular RNAs of the c-DNAs of M-MSV (MLV) and SSV

Origin of cellular RNA		Hybridization rates with M-MSV-MLV c-DNA	with SSV c-DNA
Normal human leucocytes I		1.1 %	1.75 %
Normal human leucocytes II		1.3 %	1.25 %
Human spleen I		1.0 %	2.0 %
Human spleen II		1.2 %	1.6 %
N° 31	CML	2.9 %	4.75 %
N° 47	ALL	1.4 %	1.8 %
N° 45	ALL	3.5 %	1.7 %
N° 53	ALL	0 %	14.5 %
N° 60	ALL	5.5 %	6 %
N° 64	AML	0 %	4.5 %
N° 78	AML	0 %	2 %
N° 82	ALL	2.9 %	4 %
N° 83	AML	0 %	1.75 %
N° 85	CML	0 %	1.8 %
N° 86	ALL	4.8 %	2 %
N° 89	ALL	43 %	11.5 %

a certain but not absolute correlation between the two probes with regard to the positivity or the negativity of the hybridization test. For instance, the acute myeloblastic leukemia N°64 which revealed a negative cellular RNA hybridization with the murine M-MSV(MLV) c-DNA probe showed a positive annealing of its RNA with the SSV c-DNA probe; and this was the case also with one acute lymphoblastic leukemia N°53 which in fact gave the highest rate of hybridization with the SSV probe whereas there was no positive hybridization with the murine probe. On the contrary leukemia N°86 appeared completely negative when SSV c-DNA was used as a probe and was slightly positive with the M-MSV (MLV) probe.

Unrelatedness of the sequences detected in human RNA by each c-DNA probe.

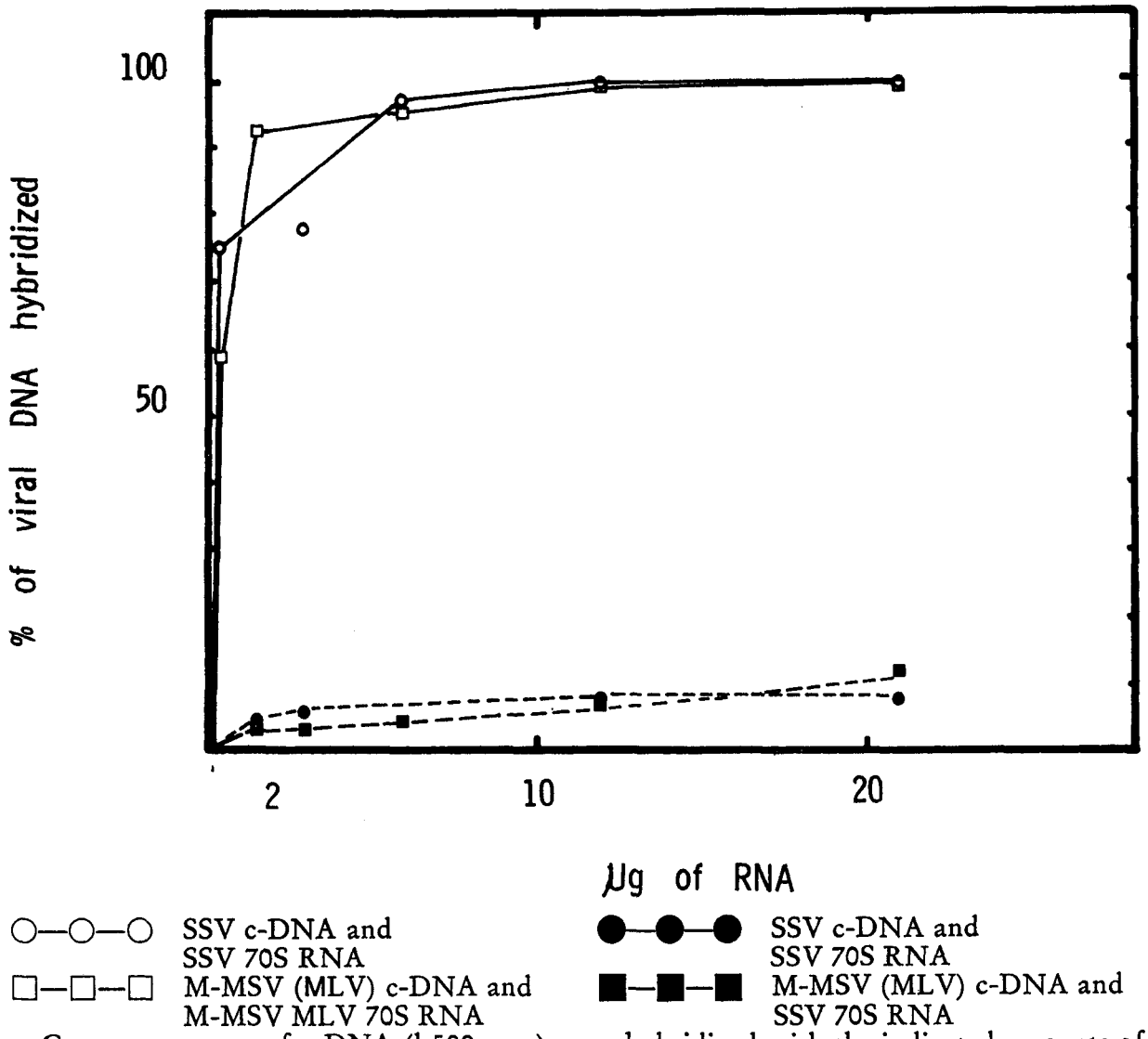
Since there was not an absolute corroboration in the results obtained by hybridizing human leukemic RNAs with the c-DNA of SSV and M-MSV(MLV),

it seemed of interest to compare the homology existing between the probes by cross hybridization experiments. Each c-DNA probe was therefore annealed with its homologous 70S RNA genome as well as with the heterogenous counterpart. Fig. 1 represents the results of this experiment and shows that there is at most 10 % homology between the two viruses. The c-DNA which is common to both viral genome was isolated after alkaline digestion of the hybrids; we failed to hybridize this c-DNA to the RNA of the acute lymphoblastic leukemia N°89 which was scored as highly positive when the entire c-DNA probes of both SSV and M-MSV(MLV) were used for the annealing tests.

Host cell information in the murine and simian viruses.

Table II shows the association rates of the simian and murine c-DNA to sheared cellular DNA of various origin. It can be seen that the SSV virus produced in

Figure 1: Cross hybridization of the nucleic acids (c-DNA and 70S RNA) of SSV and M-MSV (MLV) viruses.



Constant amount of c-DNA (1 500 cpm) were hybridized with the indicated amounts of 70S RNA for 70 hours at 68° in 4 x SSC.

Table II: Association rate of c-DNAs to cellular DNAs

Original of cellular DNA	Associated c-DNA (%)	
	MSV M (MLV)	SSV
Salmon sperm	2.2 %	3.4 %
78 A_1	100 %	93 %
SSV/NRK	15.3 %	100 %
Human spleen	11.0 %	9.2 %
Rat embryo	15.7 %	85.7 %
Mouse embryo	44 %	41.7 %

The c-DNA synthesized on M-MSV-MLV and SSV-1 virus were annealed to cellular DNAS during 70 hours at 68° in 4 x SSC. The rate of the H^3-labelled associated c-DNA was determined by S_1 Nuclease Assay.

NRK cells contained a much higher percentage of rat sequences as compared to the percentage of rat sequences (15,3 %) in the M-MSV(MLV) viruses that are produced in the 78 A-1 rat fibroblast cell line. In contrast, the proportion of human (10 %) and mouse (42–44 %) sequences are quite comparable in both viruses. It is likely however that the mouse sequences present in both viruses are not the same since there exists only 9 to 10 % homology between those virus stocks.

Discussion

Many authors advanced that some viral sequences are present in leukemic cells and that these sequences are not expressed in normal non leukemic cells (1 – 5). Corolarry results were found by Gallo and associates who reported that the c-DNA synthesized endogenously by RNA dependent-DNA polymerase in virus like particles from human acute leukemic cells was hybridizable to SSV and Kirsten sarcoma viral RNA to a higher extent than to murine leukemia viruses (8). However it was not established by these authors whether the sequences that were detected by their hybridization procedure were identical when using different viral RNA genomes. It was already observed two years ago by Benveniste and Todaro that the endogenous type C viruses of several species exhibited very little, if any, nucleic acid homology and that, among the infectious type-C viruses of different species existed a very small degree of relatedness with the noticeable exception of Kirsten mouse leukemia virus and simian sarcoma (Wooly-Monkey) or gibbon ape viruses (9). Our own experiments show clearly that the sequences detected in human ribonucleic acid from leukemic fresh cells are completely different if the simian c-DNA probe or the murine c-DNA probe are used for the molecular hybridization studies. It should be emphasized moreover than the c-DNA portion homologous to both virus genomes was unable to detect any virus related sequences in the RNA of leukemic cells, even though it was able to form stable hybrids with rat cellular DNA.

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