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Cell Fusion as a Tool for the Detection of Viral Footprints in Childhood Leukemia

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A. Introduction

In our studies on a possible role of type C oncoviruses in human leukemia we applied the technique of cocultivation of human bone marrow with an animal indicator cell line. Dog thymus A7573 cells were cocultivated with human bone marrow samples from normal individuals, leukemic patients, and nonleukemic patients. By means of the indirect cytoplasmic immunofluorescence assay (IFA), antigens which crossreacted with the major internal protein (p30) of the woolly monkey (simian) sarcoma leukemia virus (SiSV) complex could be detected (Nooter et al. 1979). In the case of childhood leukemia, five out of nine cocultures showed virus-related IFA staining.

It was hypothesized that the expression of antigens in the cocultivated A7573 cells is the result of an infectious type C virus originating from the leukemic bone marrow cells. In that case it may be expected that cell fusion instead of cocultivation would facilitate the transfer of a putative human virus to the indicator cells. We describe here our preliminary results of fusion experiments of human leukemic bone marrow with animal cell lines.

B. Materials and Methods

I. Cell Fusion

Cell fusion was essentially as described by Hales (1977). Normally, eight million nucleated bone marrow cells were mixed with two million indicator cells (either fetal dog thymus A7573 cells or rabbit cornea SIRC cells), pelleted, and fused. After fusion the cells were seeded in a 75 cm^2 Falcon. The cultures were transferred every 5 days. At each passage cells were grown on microscope slides for the fluorescence test.

II. Patient Information

Nucleated cells from bone marrow aspirates from leukemic children were obtained by sedimentation in 1% (v/v) methylcellulose in culture medium.

III. Immunofluorescence Assay

Indirect cytoplasmic immunofluorescence assays (IFA) were carried out on aceton-fixed cells as has been described previously (Nooter 1979). For IFA 50 µl of a suspension of freshly trypsinized cells ($10^5 \cdot ml^{-1}$) were transferred into wells of Teflon-coated microscope slides. The cells were incubated at 37°C for 20 h in a humidified CO_2 incubator. Thereafter the cells were fixed in aceton.

IV. Antisera

The IgG fractions of antisera were prepared according to Joustra and Lundgren (1970). The characteristics of the antisera used have been described previously (Nooter et al. 1979).

C. Results

Seven bone marrow samples from leukemic children were fused with A7573 cells and two, selected at random, with SIRC cells. The fused cultures were screened periodically for the presence of viral antigens with the IFA, using antiviral antisera directed at the major internal polypeptide [with a mol. wt. of 30,000 (p30)] of a murine type C virus (R-MuLV) and a primate type C virus (SiSV). One to five weeks after fusion antigens appeared in seven of nine cultures which predominantly reacted in the IFA with the SiSV antiserum (Table 1). The presence of antigens is a transient phenomenon: after 2 to 3 months of propagation the cultures were negative in the IFA.

Table 1. Immunofluorescence end point titers

	Antisera[a]	
	RA-SiSV-p30	RA-R-MuLV-p30
cultures[b]		
Af 91277	40	10
Af 10278	–	–
Af 51278	–	–
Af 81277	80	20
Af 10179	80	<10
Af 14879	80	20
Af 24779	40	10
Sf 23779	80	<10
Sf 14879	40–80	20
Virus positive control cultures		
REF + SiSV[c]	320–640	20–40
BALB/3T3 + R-MuLV[d]	40–80	160–320
Virus negative control cultures		
REF		
BALB/3T3, A7573		
SIRC, A204[e], NC37[f]	●—●10	●—●10

[a] Results are expressed as end point titers, presenting the reciprocal of the highest antiserum dilution with which virus-specific cytoplasmic fluorescence was still observed RA-SiSV-p30: rabbit anti-SiSV-p30; RA-R-MuLV-p30: rabbit anti-Rauscher-MuLV-p30

[b] Af and Sf cultures are fused cell cultures of A7573 and SIRC cells and leukemic bone marrow

[c] Rat embryonic fibroblasts (REF) infected with SiSV

[d] Mouse cells infected with R-MuLV

[e] Human rhabdomyosarcoma cell line

[f] Human lymphoblastoid cell line

The fused cultures have been monitored for several months for the production of extracellular reverse transcriptase. None gave positive results.

Finally, three of the fusion experiments were repeated with a HGPRT mutant of A7573 developed in our laboratory. This offered the possibility of selection for hybrid cells by growing the fused cells in so-called HAT medium (Szybalski et al. 1962). This selection did not result in higher IFA titers (they were the same or slightly lower) nor did it lead to reverse transcriptase production.

D. Discussion

The presence of type C oncovirus footprints in human mesenchymal tumors is a rather infrequent finding (for a recent review, see Nooter 1979). These observations include studies on virus polymerase, nucleic acid homology, virus antigens, antiviral antibodies, and virus isolation. The apparent low frequency of isolation of putative human retroviruses strongly suggests that these viruses are not ubiquitous in the human population. On the other hand, restricted virus expression and/or replication defectiveness could play a role as well. Subsequently, methods which facilitate virus transmission by intensive cell-to-cell contact, e.g., cell fusion in combination with sensitive detection methods, could be appropriate in studies on presumptive human viruses. In our studies described here two mammalian cell lines were fused with human bone marrow. Antigens of a possible viral origin were detected in some of these fused cultures by means of the IFA. These preliminary results show that the frequency of antigen positive cultures is comparable with the frequency found in previous cocultivation experiments (Nooter et al. 1979). However, it looks like that cell fusion is superior to cocultivation in that it speeds up

the appearance of antigens with cell passage number (1 to 5 weeks after fusion compared to 1 to 2 months after starting cocultivation). However, our cell fusion procedure never led to overt virus production. This means that the exact nature of this possible virus-related antigen has still to be determined.

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References

Hales A (1977) A procedure for the fusion of cells in suspension by means of polyethylene glycol. Somatic Cell Genet 3:227–230 – Joustra M, Lundgren H (1970) Preparation of freeze-dried monomeric and immunochemically IgG by a rapid and reproducible technique. In: Peeter H (ed) Protides of the biological fluid. Pergamon Oxford, p 511 – Nooter K (1979) Studies on the role of RNA tumour viruses in human leukaemia. Thesis, University of Leiden, pp 38–40 – Nooter K, Coolen J, Dubbes R, Zurcher C, Koch G, Bentvelzen P (1979) Type C virus antigen detection in co-cultures of human leukaemic bone marrow and dog cells. J Gen Virol 45:711–721 – Szybalski W, Szybalska EH, Ragni G (1962) Genetic studies with human cell lines. Natl Cancer Inst Monogr 7:75