VACCINIA-VIRUS-INDUCED EXPRESSION OF RETROVIRUS p30 PROTEIN IN CONTINUOUS MOUSE CELL LINES

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Increasing attention has recently been accorded to viral cocarcinogenesis, which is defined as the indirect blastomogenic effect of various agents and factors which activate specific oncogenic viruses. This phenomenon was first described by one of the authors in studies on the cocarcinogenic effects of the nononcogenic vaccinia and influenza viruses [1-3]. Subsequently, the cocarcinogenic potential of the herpesvirus was established. Studies have been conducted on the capacity of the herpesvirus to induce expression of mouse leukemia virus p30 [6, 11, 13], and to activate endogenous C-type xenotropic virus in mouse tissue culture [8, 9]. Analogous results have been obtained with the human cytomegalovirus [7, 13]. Vaccinia virus (VV) has been shown capable of activating the development of leukemia in mice [4], in inducing the expression of the retrovirus structural protein p30 in primary embryonic tissue culture of low-leukemia mice, and in activating endogenous C-type mouse virus.

This study dealt with the ability of VV to induce expression of p30, the major structural protein of retroviruses, in the cells of a number of continous mouse cell lines.

EXPERIMENTAL METHOD

The study was conducted with the following continuous lines: NclAcl 10 derived from Swiss mice 3T3 BALB clone A-31, and 3T3 BALB (an uncloned strain). The vaccinia virus consisted of a dermotropic strain (Moscow) and strain WR (USA) that had undergone two to three passages in a monolayer culture of chick embryo fibroblasts. The viruses were titrated in terms of the cytopathic effect in chick embryo fibroblast culture and plaque formation in Vero cell culture with methylcellulose overlay. The cell culture was grown on cover glasses in plastic dishes; when one day old (the monolayer was two-thirds formed), they were infected with live VV with a multiplicity of infection (MOI) of seven to eight plaque forming units (PFU) per milliliter. The cover glasses were fixed in acetone for 5-6 min after 3, 6, 9, 12, 24, 48, 72, and 96 h and examined by means of the indirect immunofluorescence technique (IIT), using goat anti-p30 MuI V-R, normal goat serum, and rabbit IgM labeled with fluorescein isothiocyanate. For positive controls we employed the following: 1) the continuous ILS-V9 cell line infected with Rauscher leukemia virus (RLV), 2) a Moloney mouse sarcoma continuous cell line (MSC), 3) cells of the continuous mouse lines under study infected with RLV in each experiment in parallel with VV infection, and 4) for comparison, NclAcl 10 cell culture infected with herpes simplex virus (HSV), type 2, strain 333. This study also utilized the immunoperoxidase technique [10, 12].

EXPERIMENTAL RESULTS

Comparison of the two VV strains showed that after two to three passages in click embryo fibroblast culture their titers did not differ significantly (7×10^7 PFU/ml for the dermotropic strain, and 2×10^8 PFU/ml for WR).

After the preliminary data had been obtained on the expression of p30 in NclAcl 10 cells under the influence of VV, a series of experiments was performed to study the dependence of p30 expression on VV MOI. The findings indicated that VV-mediated induction of p30 is MOI-dependent. Maximum p30 expression was obtained with MOI 5-7; at MOI less than 3, p30 was not detected by IIT. All subsequent experiments were conducted with MOI equal to 7.

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TABLE 1. Expression of p30 of Mouse Leukemia Virus in VV-Infected Continuous Mouse Cell Lines

VV	Post-infection time, h	Percentage of cells positive in IIT			
		NclAc110 (29-33 passages)		3T3BALB (20-24 passages)	
		anti-p30-serum	normal goat serum	anti-p30-serum	normal goat serum
Dermotropic strain (Moscow)	24	15	—	30	—
	48	25	—	40	—
	72	40	—	12	—
Strain WR (USA)	24	15	—	Unstudied	Unstudied
	48	30	—	Same	Same
	72	45	—	"	"
RLV	48	—	—	—	—
	72	15—20	—	25	—
	96	45—50	—	40—45	—

TABLE 2. Fluorescence Inhibition of Continuous Mouse Cell Lines after Specific Adsorption with Anti-p30 Serum

Anti-p30 serum	Percentage of IIT positive cells					
	NclAc110			3T3 BALB uncloned strain		
	VV (Moscow)	RLV	Uninfected	VV (Moscow)	RLV	Uninfected
Before absorption	25	45	—	40	40	—
After absorption	—	—	—	—	—	—

Note. Cell fluorescence in IIT represents results obtained after 48 h in the case of VV infection, and 96 h after RLV infection.

NclAcl 10 cells infected with live VV (both strains) showed cytoplasmic fluorescence 12 h later in IIT. Maximum fluorescence was seen in 25-40% of the cells after 48-72 h (Table 1). Cells infected with HSV fluoresced 6-8 h after infection; subsequently, in accordance with published data [11-13], fluorescence disappeared. VV-induced expression of p30 in NclAcl 10 cells was confirmed by the immunoperoxidase technique: After the addition of the Graham–Karnovsky reagent the anti-p30 treated cells acquired a distinct brown color, while the control cells were weakly stained.

The uncloned 3T3 BALB strain showed fluorescence of 30-40% of the cells 24-48 h after infection (Table 1). Normally, 8-10% of the cells of the continuous NclAcl and 3T3 BALB (uncloned strain) lines fluoresced; consequently, calculation of the percentage of fluorescent cells after VV infection was adjusted for this factor.

After 70-85 passages 3T3 BALB clone A-31 cells failed to give positive results when tested for VV-induced p30 expression.

Neutralization tests were used to confirm the specificity of cytoplasmic fluorescence of VV-treated cells of the lines under study. Cytoplasmic fluorescence was abolished by adsorbing the p30 antiserum with viable spleen cells of BALB/c mice with RLV-induced leukemia (Table 2).

Bright cytoplasmic fluorescence in IIT was obtained with 100% of the cells in the positive controls (ILS-V9 and MSC). NclAcl 10 and 3T3 BALB (uncloned strain) cells infected with RLV showed fluorescence 3-4 days after infection. Furthermore, in the latter case fluorescence was indistinct and encompassed only 30-50% of the cells.

It should be mentioned that VV actively reproduced in the culture cells in which it induced expression of p30. At the time of maximum p30 expression the VV titer approached 10^5 to $10^{5.5}$ PFU/ml. In 3T3 BALB clone A-31 cells VV reproduction was very weak.

This study demonstrated that both strains of the live VV can reproduce and elicit expression of the major retroviral structural protein – p30 – in the continuous mouse cell lines NclAcl and BALB/3T3 (uncloned strain). Lack of p30 expression in the cytoplasm of the 3T3 BALB clone A-31 cells was apparently due to the

failure of VV to reproduce intensively in these cells due to their qualitative modification as a result of spontaneous transformation. The time of appearance of cellular fluorescence after exposure to VV differed markedly from that in the herpesvirus experiment (12-24 h for VV, and 6-8 h for HSV). VV-induced expression of p30 on a temporal scale approaches the time of appearance of cellular fluorescence of RLV infected cells, i.e., the time of onset of RLV replication in these cells. The intensity and character of VV- and RLV-induced fluorescence in the IIT are quite similar. It would appear that the mechanisms of VV- and HSV-induced expression of p30 in mouse culture cells are different.

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