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STRUCTURAL ABNORMALITIES IN MURINE LEUKEMIA VIRUSES PRODUCED BY INTERFERON-TREATED CELLS

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Summary: The infectivity of Moloney murine leukemia virus (MMLV) particles derived from interferon-treated mouse TB cells is very low. There is a defective viral protein-protein complex between viral p30 antigen and reverse-transcriptase in MMLV produced by interferon-treated TB cells. This defect may account in part for the decreased infectivity of these virus particles.

INTRODUCTION: Interferon treatment is thought to inhibit virus replication by inhibiting the initiation or elongation steps of virus protein synthesis (1), although there is also some evidence that virus-directed transcription is delayed by interferon pretreatment (2); however, studies on the effect of interferon treatment on the replication of murine leukemia viruses (MLV) suggested that the site of inhibition was not directly related to protein synthesis. Early reports indicated that interferon treatment inhibited a late step in the assembly of MLV; this resulted in an inhibition of virus budding and thus an increase in the concentration cell-associated viral RNA and antigens (3,4). In other systems, however, although production of MLV particles was almost equal to that of untreated cells, the infectivity of the particles from interferon-treated cells was very low (5,6,7). One possible basis for this low infectivity was the presence in moderate concentration of a glycoprotein of about 85,000 in molecular weight (gp85) in MLV from interferon-treated cells; this protein was only a trace component in MLV produced by control cells (8). In the present study we report the presence of

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a defective virus protein-protein complex between viral p30 antigen and reverse-transcriptase in Moloney MLV(MMLV) produced by interferon-treated cells (9).

RESULTS: In searching for possible structural abnormalities in MMLV derived from interferon-treated cells we noted a difference in primer-template activities. In virus pools containing approximately the same concentration of virus (as evidenced by the concentrations of group specific or p30 viral antigen) there was, in MMLV from interferon-treated cells, a decrease in reverse transcriptase activity with 70S viral RNA as a template; however, no significant decrease was observed with $(dT)_{12}.(rA)_n$ as a template (Table 1). In an attempt to determine the cause of the decreased activity with the 70S viral RNA as a primer template, the status of a p30-reverse transcriptase complex was examined.

Such an 18S multiprotein complex, containing reverse transcriptase and ribonuclease H activities together with the major internal protein of the virus, p30, and 4S RNA, has been reported (9) in a study on Rauscher leukemia virus (RLV). The reverse transcriptase-p30 complex stimulated viral DNA synthesis by 2 to 3-fold over that seen with purified reverse transcriptase alone, when 70S RNA from RLV was used as a template; however, no difference in the rate of DNA synthesis could be seen between the purified enzyme and the complex when the artificial $(dT)_{12}.(rA)_n$ template was used (9). It seemed possible that the decrease in enzyme activity associated with interferon treatment (Table 1) might be related to inefficient complex formation between p30 and the reverse transcriptase.

We therefore analyzed detergent-treated MMLV on 5-20% glycerol gradients (Figs 1) in an attempt to isolate the complex. For comparative purposes detergent-treated RLV from which the complex was originally obtained was also analyzed (9). As shown in Fig 1C, an approximately 18S reverse transcriptase-p30 complex was present. Enzyme activity and p30 antigen were also present at the top of the gradient, probably in a non-complexed form.

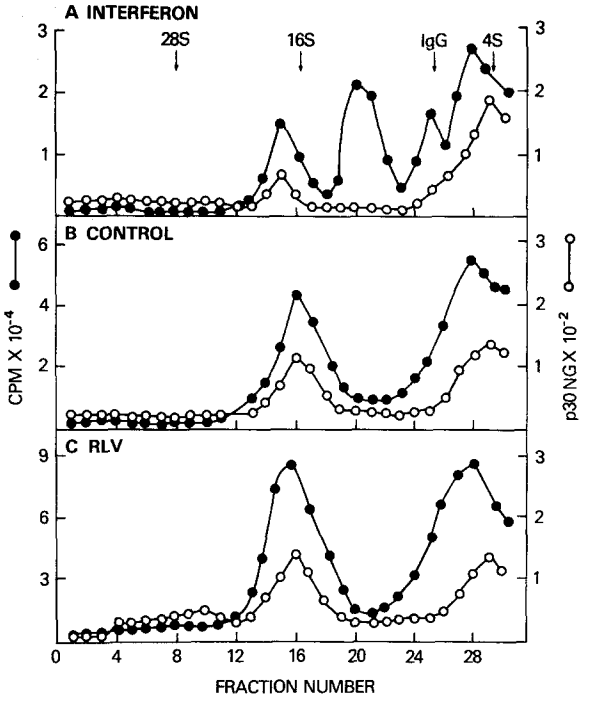

Figure 1. Triton X-100 was added to the virus samples described in Table I from interferon-treated (A), or control, (B) TB cells, or to Rauscher leukemia virus (RLV), (C) to a final concentration of 0.05%, v/v in the presence of 1 mM dithiothreitol and 0.34 M KCl. The disrupted virion preparation was kept in ice for 15 min. and then centrifuged at 27,000 x g for 10 min. The solubilized material was layered on a 4.5 ml., 5-20% glycerol gradient in 10 mM Tris.HCl buffer, pH 7.4, containing 0.1 M NaCl and centrifuged in a Beckman L-2 75B ultracentrifuge at 65,000 rpm in SW 65 rotor for 2.5 hours. Fractions (200 ul) were collected by puncturing the bottom of the tube and assayed for DNA polymerase activity (9) using $(dT)_{12}.(rA)_n$, or for p30 antigen (12). The sedimentation markers are indicated at the top.
●—● counts per min x 10^{-4} of ^{3}H TMP incorporated in assay for reverse transcriptase activity; o—o p30 antigen in nanograms (NG) x10^{-2}.

Gradients of lysed MMLV from untreated cells (Fig 1B) resembled those from purified RLV (Fig 1C) in that a large portion of both p30 and reverse transcriptase activity cosedimented, presumably in a complex. In gradients containing MMLV from interferon-treated cells, complex formation was greatly reduced (Fig 1A) and most of the p30 activity was found at the top of the gradient while peaks of reverse transcriptase activity were located in several regions of the gradient, but in only one (about 18S) did it cosediment with p30. Thus, although the reverse transcriptase-p30 complex was present in the

Table I. Reverse Transcriptase Activity of Moloney Murine Leukemia Virus from Interferon-Treated or Control Cells.

		Treatment of Cells from which MMLV was derived:	
		None	Interferon
p30 antigen (ng/mg protein)		97	88
Reverse transcriptase activity			
Template	Deoxynucleotide incorporated		
70S RLV-RNA	^{3}H d-GMP	350	78
$(dT)_{12}.(rA)_n$	^{3}H TMP	5000	3500

Virus from control or interferon-treated (200 units/ml) cells was concentrated by sedimentation at 40,000 x g for 90 min. The virus was resuspended in TES (Tris.HCl, pH 7.2, 10mM, NaCl, 100mM, and EDTA, 1mM) and layered over a 20-60% sucrose gradient in TES. The gradients were sedimented at 100,000 x g for 14 h in a Beckman SW 27 rotor and the virus band at a density of 1.15 to 1.17 g/ml was collected, diluted with TES and sedimented at 40,000 x g for 90 min., and the final virus pellet resuspended in 250 ul of TES. Aliquots of the virus samples were assayed for p30 antigen (12) and reverse transcriptase activity using 70S RNA of Rauscher leukemia virus (70S RLV-RNA) or $(dT)_{12}.(rA)_n$ as templates (9). Activities of the reverse-transcriptase are given in picomoles of precursor incorporated into acid precipitable DNA.

virus from interferon-treated cells, its concentration was approximately 6-fold less than in virus from control cells. It is important to reemphasize that while the total amount of enzyme activity present in the whole preparations derived from interferon-treated cells was very significantly decreased with RLV 70S RNA as a template, there was only a moderate decrease with $(dT)_{12} \cdot (rA)_n$ as a template (Table 1). This is probably because uncomplexed enzyme was able to use the artificial template almost as well as complexed enzyme (9). In the experiments summarized in Fig 1, $(dT)_{12} \cdot (rA)_n$ was used as a template so that all of the activity present could be demonstrated. Thus, although the total amount of activity present in the gradient was not greatly decreased, its location was shifted.

DISCUSSION: These and previous results (8) suggested that there are at least two abnormalities in the structural proteins of MMLV from interferon-treated cells. The first is a defect in the gp85, so that its cleavage in the virion is somewhat inefficient. The other abnormality is in the formation of the p30-reverse transcriptase complex which has been postulated to be important in virus assembly (9). Studies are currently in progress to establish the relationship of these abnormalities to the interferon-induced inhibition of viral infectivity. Since it is doubtful whether any single one of the structural abnormalities discussed could alone account for the thousand-fold inhibition of MMLV infectivity observed in virus from interferon-treated cells, it is possible that a combination of these abnormalities (together with others which may exist) might together give rise to the observed deficiency in infectivity. Combinations of such structural abnormalities might account for the differing reports of the locus of interferon action on MLV replication in various virus-cell systems. These include: (i) a decrease in the number of virus particles formed (13); (ii) inhibition of virus particle release from the plasma membrane (14,15); and, (iii) production of virus particles with a marked decrease in infectivity (5,6,7). It seems reasonable that such varied sites of inhibition might well be related to differing expression of a series of structural abnormalities in murine leukemia virus proteins.

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