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ASSEMBLY OF XENOTROPIC MURINE LEUKAEMIA VIRUS-RELATED ANTIGENS FROM THE SURFACE OF MOUSE L CELLS BY VESICULAR STOMATITIS VIRUS

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Summary. — Two xenotropic murine leukaemia virus (XMuLV)-related proteins — a major envelope glycoprotein gp70 and a 90K protein (probably corresponding to the uncleaved envelope precursor) — were expressed on the surface of mouse L cells as demonstrated by lactoperoxidase-catalysed iodination and immunoprecipitation with anti-XMuLV serum. These two proteins out of many labelled cell surface proteins were selectively incorporated into vesicular stomatitis virus (VSV) virions. Significant differences were found in the amounts of labelled XMuLV-related proteins between L cells and two cell lines infected with XMuLV (rabbit SIRC and lamb LKC cells). The two viral antigens represented only a small proportion of radioactivity on L cells. While in XMuLV-infected SIRC and LKC cells, the gp70 was the major labelled surface protein no detectable amounts of XMuLV-related 90K protein or of cell-specific proteins were found in these cells.

Key words: *xenotropic murine leukaemia virus*; *vesicular stomatitis virus*; *L cells*; *surface labelling*; *immunoprecipitation*; *polyacrylamide gel electrophoresis*

Introduction

VSV reproduced in mouse L cells contains no significant proportion of pseudotype particles which are detected as infectious virions resistant to anti-VSV serum. However, probably 100% of VSV virions reproduced in L cells acquired at least one or few MuLV-related molecules, as can be demonstrated by specific immunoprecipitation with antibodies and *Staphylococcus aureus* cells (Závada *et al.*, 1983). The cells produce type C virions and contain MuLV related antigens. However, it has not been cleared if also the gp70 is present among them (Kinding *et al.*, 1968; Schäfer *et al.*, 1972). In fact, the reactions used probably detect mainly the internal structural MuLV proteins.

The aim of this work was to provide (using surface labelling and immunoprecipitation) answers to the following questions:

1. What is the qualitative and quantitative representation of XMuLV-related surface antigens in L cells as compared to other cells which were infected with XMuLV and can give rise to VSV pseudotype?
2. How efficiently does VSV assemble the XMuLV-related antigens present on the surface structure of L cells?

The results obtained can explain the above mentioned observations with phenotypic mixing of VSV in L cells. In addition our work showed that certain subsets of L cell surface proteins selectively assembled by VSV (Lodish and Porter, 1980) are viral (XMuLV-related) in origin. This observation supports the hypothesis (Závada, 1982) that enveloped viruses such as VSV may efficiently assemble surface glycoproteins of unrelated viruses but not cellular proteins.

Materials and Methods

Viruses, cells and media were the same we used before (Závada *et al.*, 1977; 1983).

Antiserum preparation to XMuLV was described in accompanying paper (Závada *et al.*, 1983).

Virus purification. Virus from tissue culture medium was passed through a 0.45 u Millipore filter, concentrated by high speed spin and purified by equilibrium centrifugation in 10 to 55% sucrose gradient (Beckman L5.75, SW27, 27.000 rev/min, 4 hr).

Iodination of surface proteins was done in monolayer cultures. Cells were rinsed three times with phosphate buffered saline, pH 7.2 (PBS). PBS was then added (0.5 ml/5 cm dish) followed by carrier-free $Na^{125}I$ (usually to a final concentration of 7.4 MBq/ml). The reaction was initiated by addition of glucose (to 5 mmol/l), lactoperoxidase (EC 1.11.1.7) and glucose oxidase (EC 1.1.3.4.) to final concentrations of 20 μg/ml and 4 μg/ml, respectively. Reaction was allowed to continue for 20 min at room temperature with occasional swirling and labelling was stopped by addition of 0.05 mmol/l tyrosine. To isolate surface proteins (either before or after infection) monolayers were rinsed three times in PBS and then 0.5 ml of a solution containing 0.05 mol/l Tris-HCl pH 7.4, 25 mmol/l KCl, 5 mmol/l $MgCl_2$, 5 mmol/l $CaCl_2$, 0.5% Nonidet P-40, soya bean trypsin inhibitor (10 μg/ml), 1% saturated ε-aminocaproic acid, N-tosyl-L-lysine-chloromethyl (10 μg/ml) and phenylmethyl sulphonylfluoride (1 mmol/l) was added. Solubilized proteins were used either directly or after immunoprecipitation in sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis (PAGE).

Immunoprecipitation. Reaction mixture of (^{125}I) L-cell extract and VSV propagated in surface-labelled L cells (and subsequently purified) contained : 1 ml PBS, 1%, foetal calf serum (FCS) and 5 μl of anti-XMuLV or control serum. Incubation was 1 hr at room temperature. In some experiments, the sera were preabsorbed with normal human cells and either with normal mink cells or with XMuLV infected mink cells (usually 5 μl of serum was preabsorbed with 5×10^6 of human cells and 20×10^6 of mink cells). Cells used for absorption were washed with 1% FCS in PBS and resuspended in the same solution. Immune complexes were precipitated with a 10-fold excess of *Staphylococcus aureus* suspension in PBS containing 1% Triton-X-100, washed three times in the same buffer and finally eluted with sample buffer (Laemli, 1970) for 5 min at 100 °C).

SDS-PAGE. Slab gels containing a 10% of acrylamide were prepared in a discontinuous Tris-HCl-glycine buffer as described by Laemli (1970). Autoradiographs were made with Medix Rapid X-Ray film (Czechoslovakia), using intensifying screens at —70 °C.

Results and Discussion

XMuLV-related proteins expressed on the surface of mouse L cells

The enzymatic iodination technique was used to detect XMuLV-related antigens on the surface of mouse L cells. SDS-PAGE of solubilized L cell proteins revealed many labelled bands ranging from 10,000 – 100,000 daltons (Figs. 1 and 2, lanes 1 and 7). The bulk of radioactivity, however, is seen in the front of migration (low m.w. proteins and glycolipids, lanes 1 and 7). Anti-XMuLV serum (preabsorbed with normal human embryo cells as well as with mink cells) precipitated from such (^{125}I)-L cell extract two labelled proteins with apparent m.w. 70K and 90K, respectively (Figs. 1 and 2, lanes 2 and 8). In the next experiment, the same serum was preabsorbed with normal human embryo cells together with XMuLV-infected mink cells. As expected, such absorption abrogated the precipitation of 70K and 90K proteins from the iodinated cell extract (Figs. 1 and 2, lanes 3 and 9). No labelled proteins have been found in SDS-PAGE after immunoprecipitation with non-immune rabbit serum preabsorbed as described above (Figs. 1 and 2, lanes 4, 10 and 11). In a parallel experiment (not shown), the purified XMuLV virus was labelled under the same conditions as L cells. The most heavily labelled viral band corresponding to major gp70 envelope glycoprotein was precipitated with anti-XMuLV serum preabsorbed with normal human embryo cells as well as with mink cells; it was not precipitated with the serum preabsorbed with normal human embryo cells together with XMuLV-infected mink cells and with non-immune rabbit serum.

From our results we conclude that at least two XMuLV-related proteins are present on the surface of mouse L cells, namely the major envelope gp70 and a protein of m.w. 90K, probably corresponding to the uncleaved envelope precursor.

VSV grown in L cells selectively assembles XMuLV-related proteins

Monolayers of L cells were surface labelled with lactoperoxidase catalysed iodination (as above) and then infected with VSV at multiplicity of 10 PFU/cell. The progeny virus was purified and used in the following analyses. The lanes 5 and 12 (Figs. 1 and 3) show that two labelled proteins with apparent m.w. 70K and 90K, respectively, are incorporated into VSV (distribution of radioactivity in these two bands varied, however, from experiment to experiment). This result as well as the different pattern of the total labelled cell surface proteins (lanes 1 and 7) confirm the earlier reports by Lodish and Porter (1980). It appears therefore that VSV assembles only the 70K and 90K proteins out of the numerous labelled cell surface proteins. The striking similarity of lanes 6 and 2 (or 8) suggests that the two L cell proteins (70K and 90K) incorporated into VSV virions are probably the XMuLV-related antigens. The analysis shown in lanes confirm that 70K and 90K proteins are selectively assembled by VSV from L cells (Figs. 2 and 3, lanes 12 and 13) since anti-XMuLV serum which had been absorbed with control human cells and with XMuLV-infected mink cells lost the capacity to precipitate any radioactive material from solubilized VSV grown in surface-clabelled L cells (Fig. 3, lane 15). This further demonstrates that the L cell yurface proteins assembled by VSV are XMuLV-related.

Lodish and Porter (1980) also reported that VSV incorporated selectively cellular polypeptides following infection of Vero cells. Since no retrovirus has yet been isolated from African green monkey (Vero cell donor), no corresponding serum is available; therefore, the experiments which could identify the origin of Vero-derived VSV antigens cannot be done.

High expression of XMuLV proteins on the surface of persistenty infected cells

After enzyme-catalysed iodination of the rabbit SIRC and lamb LKC cells persistently infected with XMuLV (in contrast to uninfected parallel cultures) most of the radioactivity in SDS-PAGE is found in a single band corresponding to the major envelope glycoprotein gp70 (Figs. 4 and 5, lanes 19 and 24). No distinct zone was seen in the region of envelope precursor, even after immunoprecipitation of labelled cell extracts with anti-XMuLV serum. SIRC and LKC cells differed from the L cells by a higher expression of gp70 on the cell surface and by absence of the uncleaved envelope precursor (as detected under the conditions of our experiment).

The small amounts of XMuLV gp70 on the surface of L cells (when compared to XMuLV-infected SIRC and LKC cells) might be the reason for the failure to detect VSV pseudotypes resistant to anti-VSV serum in the L cells.

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Explanation of Figures (Plates XI—XIII):

SDS-PAGE of the immunoprecipitated material.

Material	Precipitation with	Serum preabsorbed with	Figure
1 — ^{125}I-L cells	O = total	O	
2 — ^{125}I-L cells	anti-XMuLV	HE + M	
3 — ^{125}I-L cells	anti-XMuLV	HE + MX	1
4 — ^{125}I-L cells	non-immune	HE + M	
5 — VSV from ^{125}I-L cells	O = total	O	
6 — VSV from ^{125}I-L cells	anti-XMuLV	HE + M	

7 — ^{125}I-L cells	O = total	O	2
8 — ^{125}I-L cells	anti-XMuLV	HE + M	
9 — ^{125}I-L cells	anti-XMuLV	HE + MX	
10 — ^{125}I-L cells	non-immune	HE + M	
11 — ^{125}I-L cells	non-immune	HE + MX	
12 — VSV from ^{125}I-L cells	O = total	O	3
13 — VSV from ^{125}I-L cells	anti-XMuLV	O	
14 — VSV from ^{125}I-L cells	anti-XMuLV	HE + M	
15 — VSV from ^{125}I-L cells	anti-XMuLV	HE + MX	
16 — VSV from ^{125}I-L cells	non-immune	O	
17 — VSV from ^{125}I-L cells	non-immune	HE + M	
18 — VSV from ^{125}I-L cells	non-immune	HE + MX	
19 — ^{125}I-XMuLV inf. SIRC cells	O = total	O	4
20 — ^{125}I-XMuLV inf. SIRC cells	anti-XMuLV	HE + M	
21 — ^{125}I-XMuLV inf. SIRC cells	non-immune	HE + M	
22 — ^{125}I-XMuLV inf. SIRC cells	anti-XMuLV	HE + M + SIRC	
23 — ^{125}I-XMuLV inf. SIRC cells	non-immune	HE + M + SIRC	
27 — ^{125}I-XMuLV inf. LKC cells	O = total	O	5
25 — ^{125}I-XMuLV inf. LKC cells	3ti-XMuLV	HE + M	
26 — ^{125}I-XMuLV inf. LKC cells	non-immune	HE + M	
27 — ^{125}I-XMuLV inf. LKC cells	anti-XMuLV	HE + M + LKC	
28 — ^{125}I-XMuLV inf. LKC cells	non-imune	HE + M + LKC	

HE = human, embryo cells; M = MINK cells; MX = XMuLV infected MINK cells