A LARGE GLYCOPROTEIN OF MOLONEY LEUKEMIA VIRUS DERIVED FROM INTERFERON-TREATED CELLS

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Summary: Moloney murine leukemia virus produced in interferon-treated mouse thymus and bone marrow cells has a high particle to infectivity ratio (1, 2). This virus contains a prominent glycoprotein with a molecular weight of about 85,000. This large glycoprotein is only a very minor component of Moloney leukemia virus produced in control TB cells and may be an uncleaved precursor to gp 69-71 (3, 4).

INTRODUCTION: In interferon-directed inhibition of most virus infections, there is an inhibition of viral RNA and protein synthesis (5). Interferon treatment of murine leukemia virus infected cells does not result in inhibition of the synthesis of several virus proteins which have been studied (6, 7, 8, 9). Transmission (10) and scanning electron micrographs (11) of these cells have indicated that the number of cell-associated virus particles was not decreased, and in some cases was increased after interferon treatment. In other systems virus particle production appeared to be almost normal but the virus produced had a marked decrease in infectivity (1, 2, 12). In the present study, the glycoproteins of low infectivity Moloney leukemia virus produced in interferon-treated TB cells was studied (1, 2). A large protein with a molecular weight of 85,000 was a promininent constituent of this virus; this glycoprotein was found in only trace amounts in virus derived from control TB cells.

MATERIALS AND METHODS: Cells and Virus: TB a fibroblastic cell line established from mixed cultures of bone marrow and thymus CFW/D mouse cells (13) was adapted to McCoy's 5a medium (Grand Island Biological Company, Grand Island, New York) containing 10% heat-inactivated fetal bovine serum, penicillin (100 u/ml), streptomycin (100 µg/ml), fungizone (25 µg/ml) and gentamicin (50 µg/ml). A chronic virus producing TB cell line was established by infection with wild type Moloney murine leukemia virus (MMuLV). Cells from passages 5 through 15 after MMuLV infection were used for virus collections. MMuLV-infected TB cells were provided by Dr. P. K. Y. Wong, University of Illinois, Urbana, Illinois.

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Interferon: Mouse interferon was partially purified on an immunoadsorbant column in Dr. K. Paucker's laboratory at the Pennsylvania Medical College (14). The preparation used has a specific activity of at least 2 x 10^7 international reference units per mg of protein and its antiviral activity had the chemical and physical properties usually ascribed to interferon (15).

Virus Production, Concentration and Purification: In MMuLV infected TB cells treatment with interferon at 200 units/ml for 36 hours gave an inhibition of virus yield of 75% measured by reverse transcriptase activity and $\geq$ 99% assayed by viral infectivity.

Subconfluent TB cultures chronically infected with MMuLV were treated with 200 units/ml of interferon for 36 hours. After this treatment, tissue culture fluid was harvested every 4 hours and flasks were refed with interferon-containing medium. The fluids were harvested when monolayers exhibited 80-100% confluency.

Tissue culture fluids were clarified at 10,000 x g for 15 minutes and then pelleted at 40,000 x g for 80 minutes. The virus pellet was resuspended in TES (10 mM Tris, pH 7.4, 1 mM EDTA, 100 mM NaCl) and overlayed onto a 20-60% continuous sucrose gradient in TES. The gradients were centrifuged at 183,000 x g for 5 hours in a Beckman SW 41 rotor.

The virus band at a density of 1.15-1.17 g/ml was collected, diluted gradually with TES, and again sedimented at 40,000 x g for 90 minutes and the final virus pellet resuspended in a small volume of TES and lyophilized.

Internal Labeling of Virus: Control and interferon-treated MMuLV-infected TB cells were grown in roller bottles and 24 h after the addition of interferon in low glucose-containing (25% of the normal level) McCoy's 5a medium, [^{3}H]-glucosamine (10 µCi/ml, specific activity 20.7 Ci/mmole New England Nuclear, Boston, Massachusetts) was added. Twelve to sixteen hours after addition of radioactive glucosamine, culture fluids were harvested and the flasks refed. Fluids from interferon-treated or control cells were processed as described in previous paragraphs.

Surface Labeling of Virus: Sucrose gradient purified virus was oxidized with mild periodate treatment, by addition of 0.1 ml of 0.1 M sodium metaperiodate into 2.5 ml of purified virus in TES (16, 17). After 10 minutes at 23°C with frequent agitation, the reaction was terminated by adding 35 ml of cold TES. The virus was then concentrated by sedimentation at 40,000 x g for 90 minutes, and washed twice with TES. The final washed virus pellet was resuspended in 1 ml of TES and 200 µl of TES containing 3 mCi of [^{3}H]-sodium borohydride (specific activity 16 Ci/mmole, Amersham Searle Corporation, Arlington Heights, Illinois) was added. The mixture was incubated at room temperature for 30 minutes with occasional shaking. The virus was again sedimented by centrifugation at 40,000 x g and washed twice with cold TES. The final virus pellet was resuspended in a small volume of TES and lyophilized.

Gel Electrophoresis: Tris-buffered-SDS gradient polyacrylamide slab gels (7.5-16%) were prepared as described by Laemmli (18). Gels electrophoresed in a slab gel apparatus (Hoefer Scientific, San Francisco, California) were stained with Coomassie brilliant blue to locate the molecular marker proteins, phosphorylase A (95-100K), bovine serum albumin (68K). After destaining, gels treated with dimethyl sulfoxide-PPO according to Bonnar and Laskey (19) and dried, were exposed to X-OMAT X-Ray film (Kodak).

RESULTS: Two methods were used to label the viral glycoproteins: (a) incubation of infected cells with [^{3}H]-glucosamine as a precursor and purifying the radioactive virus produced; or (b) treating purified virus with sodium metaperiodate and then labeling with tritiated sodium borohydride. In both

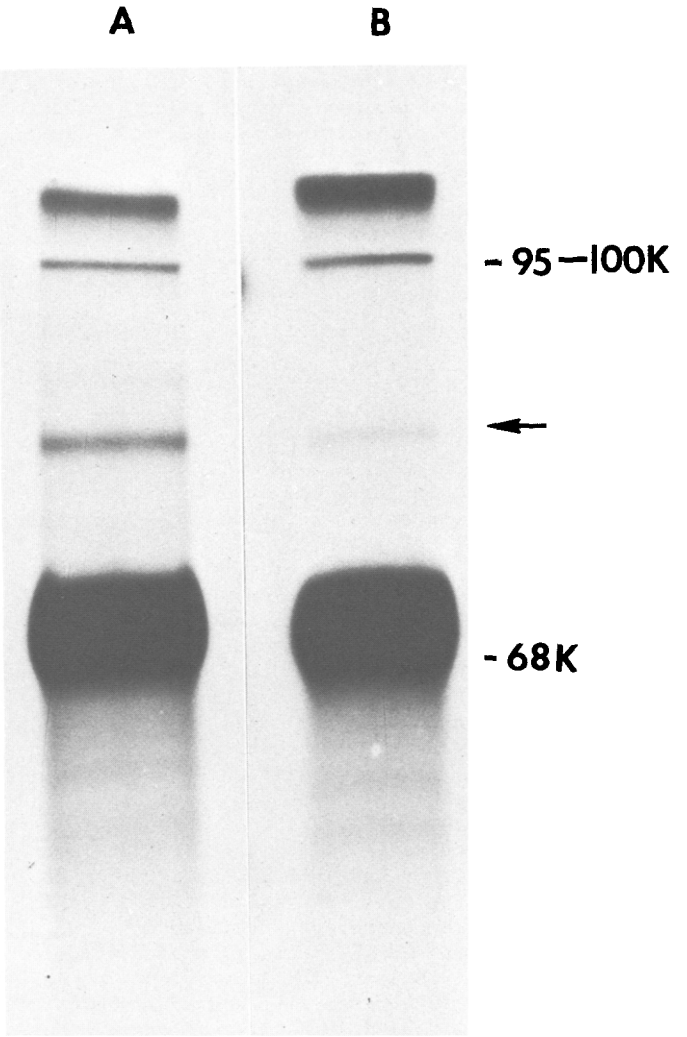

Figure 1. Analysis of viral glycoproteins of purified MMuLV derived from interferon-treated and control TB cells.

MMuLV-infected TB cells treated with interferon (200 units/ml) for 24 hours were labeled with [^{3}H]-glucosamine for 12-16 hours. Extracellular virions from control (1B) or interferon-treated cultures (1A) were concentrated, purified, and analyzed by electrophoresis in a 7.5-16% Tris-buffered SDS-gradient polyacrylamide slab gel. The gel was PPO-impregnated, dried, and exposed to X-OMAT R film. Phosphorylase A (95-100K) and bovine serum albumin (68K) were used as molecular marker proteins. The arrow indicates the mobility of a glycoprotein of apparent molecular weight of approximately 85,000 dalton.

cases the viral proteins were then prepared for analysis by electrophoresis on SDS-gradient polyacrylamide slab gels (7.5-16%). The same number of counts was put in each slot. Under the conditions employed only glycoproteins were radioactive.

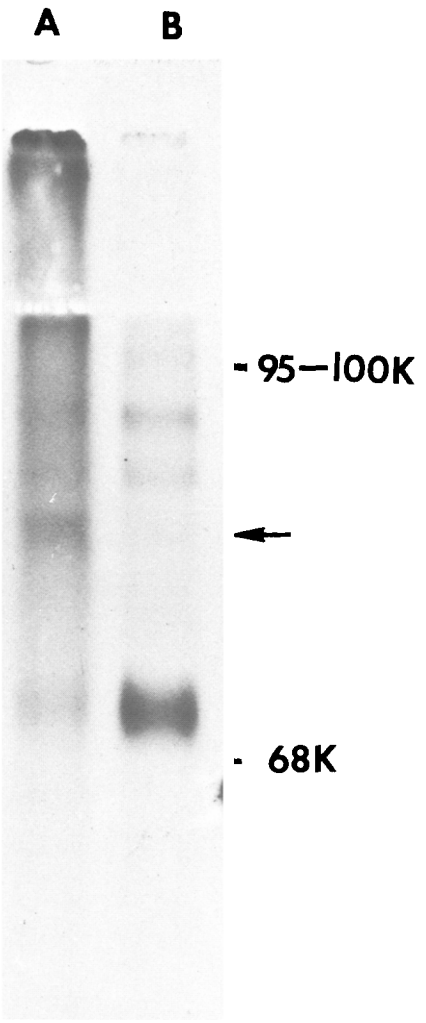

Figure 2. SDS-gradient PAGE patterns of surface-labeled viral glycoproteins of MMuLV derived from interferon-treated and control cells.
Purified virions sequentially treated with sodium metaperiodate and [^{3}H]-sodium borohydride were analyzed as described in Figure 1. (A)-interferon-treated, (B)-control.

In the virus labeled with [^{3}H]-glucosamine there was present a prominent band of glycoprotein with a molecular weight about 85,000 in virus derived from interferon-treated TB cells (Figure 1A) while only a trace amount of this glycoprotein was present in virus derived from control cells not treated with interferon (Figure 1B). In the case of virus tritiated after periodate

treatment, a prominent glycoprotein component with a molecular weight of 85,000 was also present in virus from interferon-treated cells (Figure 2A). Again this glycoprotein was only present in trace amounts in virus produced by the control cells (Figure 2B).

DISCUSSION: These findings indicate that the 85,000 dalton protein found in increased amounts in MMuLV virus derived from interferon-treated TB cells was a glycoprotein (hereafter designated as gp85) because it contained glucosamine and was oxidized by periodate treatment of the virus. The latter also indicated that gp85 was located in the outer portion of the viral membrane.

Previously we have shown that although virus derived from interferon-treated TB cells had decreased infectivity per particle this was not due to any qualitative alteration in the RNA of the virus (2). Viral lipids were also probably not altered as the ganglioside content of virus from interferon-treated cells was identical to that of virus from control cells (L. Kohn, E. Grollman, E. Chang, R. Friedman, manuscript in preparation). Therefore, the marked increase in the fraction of viral protein made up by gp85 in virus from interferon-treated, as compared to virus from control cells, is the only difference we have so far found to account for the lowered infectivity of the former.

The origin of gp85 in MMuLV is uncertain but it may well be related to a glycoprotein precursor of the major viral glycoprotein, gp69/71; this precursor, like gp85, has a molecular weight of approximately 85,000 (3, 4). If, indeed, the origin of the excess gp85 in virus derived from interferon-treated cells is an uncleaved precursor to gp 69/71, one effect of interferon treatment in this system must be to inhibit cleavage processing of a viral glycoprotein.

An alteration in such a fundamental process as cleavage of viral proteins might account for the unusual inhibitory mechanism of interferon action on the production of murine leukemia viruses. The partial impairment of cleavage in turn may be due to interferon-induced alterations in the

chemical (20), physical (21), and morphological (21) characteristics of the plasma membrane which have been demonstrated to take place in interferon-treated cells. In TB (1, 2) or some AKR systems (12) these alterations may result in the release of a virus with decreased infectivity because of the impaired cleavage of a gp85 precursor. In other systems studied (6, 7), the interferon-induced alteration in the plasma membrane may be significant enough to impair even the release of virus from the cell.

In mouse cells chronically infected with Rauscher murine leukemia virus, immunoprecipitation of several intracellular, virus-specific proteins (p15, p30 and gp69/71) has revealed that interferon treatment affected neither the synthesis nor the cleavage of these proteins (22). The concentration of interferon used, strongly inhibited the release of virus particles. However, quantitative differences of the magnitude observed by gel electrophoresis of proteins from purified virus particles may be undetectable by immunoprecipitation of total intracellular virus-specific proteins.

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