Synthesis of Murine Leukemia Virus Proteins Differentiating Friend Erythroleukemia Cells

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ABSTRACT

In agreement with previous observations, the Friend 745 line of virus-induced erythroleukemia cells can be induced by dimethyl sulfoxide to synthesize hemoglobin and to increase simultaneously their content of morphologically recognizable virus particles, especially in cytoplasmic vesicles. In apparent contrast to the electron microscopic results, the cellular content of the major murine leukemia virus (MuLV) proteins p30 (the major internal core protein) and gp70 (the envelope glycoprotein) were measured by radioimmunoassay and were found to be approximately the same in control and in induced cells. Control and induced cells are also approximately as active in the synthesis of the protein precursors of p30, reverse transcriptase, and gp70 in the processing of these precursors by proteolysis and glycosylation. Furthermore, the noninfectious MuLV synthesized by this cell line is released into the culture medium to the same extent by control and induced cells. Although some of the virions formed in induced Friend 745 cells bud into cytoplasmic vesicles that are absent from control cells, this intravesicular budding is not associated with a major increase in the synthesis, processing, or cellular content of virion proteins.

MuLV synthesis by Friend 745 cells was compared with that of Ostertag-derived F46/K erythroleukemia cells that produce infectious virus. The synthesis and processing of MuLV-specific proteins were very similar in these independently isolated cell lines, despite the major difference in infectivity of the virus produced.

INTRODUCTION

Erythroleukemia cells from the spleens of mice infected with Friend virus have been established as permanent cell cultures (8, 10, 20, 30). Several of these permanent cell lines can be induced by dimethyl sulfoxide and other inducers to synthesize hemoglobin (10, 20, 30) and to undergo a developmental process that morphologically resembles normal erythropoiesis (24). In addition, some other erythrocyte-specific proteins are coinduced with hemoglobin (6, 12, 14, 15). As in normal erythropoiesis, rRNA synthesis occurs only during the earliest developmental period, and the cellular RNA content and protein synthesis activity subsequently decline (28).

Sato et al. (24) examined Friend leukemia cells by electron microscopy and observed that the cells that had been induced to synthesize hemoglobin contained numerous cytoplasmic vesicles filled with C-type virus that were absent from control cells. Furthermore, there appeared to be an increase in C-type particles budding from the plasma membrane of the induced cells. These striking observations were largely confirmed by our own electron microscopic studies with Friend 745 cells and also in our laboratories (19, 30, 33). These observations have been interpreted (24) as suggesting either that MuLV synthesis is coinduced with hematopoiesis in Friend leukemia cells or that the release of assembled MuLV's is inhibited in induced cells so that the virus particles accumulate intracellularly.

Since cellular differentiation is believed to be an important factor in regulating expression of endogenous and exogenous C-type retroviruses (3), we decided to analyze more thoroughly the synthesis of MuLV in the cultured Friend 745 cell line. Our results show that MuLV proteins are synthesized and processed as actively in uninduced cells as in induced cells.

MATERIALS AND METHODS

Cell Culture. Friend clone 745 was obtained from the Genetic Mutant Cell Culture Repository. The F4-6 cell line was generously supplied by W. Ostertag (Max Planck Institute, Gottingen, Germany). We now designate the line grown in this laboratory for many generations as F4-6/K cells. The cells are grown in suspension as described in detail elsewhere (15, 27). For induction with dimethyl sulfoxide, successive 2-fold dilutions of a stationary-phase culture with fresh medium were made at 0, 24, and 48 hr. At these times, the induced cultures were made successively 1, 1.5, and 2% in dimethyl sulfoxide.

MuLV Assay. The titer of MuLV in the culture fluid was measured with a modification (27) of the sarcoma-positive, leukemia-negative method of Bassin et al. (4). Titers of MuLV within the cells were determined as follows. Cells were sedimented at 2,000 × g for 10 min at 2° and were washed once with an equal volume of cold Dulbecco's phosphate-buffered saline (Grand Island Biological Co., Grand Island, N. Y.). The cells were resuspended in 2 ml of a buffer containing 10 mM Tris-HCl, pH 7.4:10 mM KCl:1.5 mM MgCl2 and were homogenized in a Dounce-type tissue grinder with 50 strokes of the "tight" pestle and 30 strokes of the "loose" pestle. This homogenate was used in the sarcoma-positive, leukemia-negative assay.

Determination of Virion Release. From 76 to 96 hr of the
induction protocol, control and induced Friend 745 cell cultures were supplemented with \[^{3}H\]leucine, 28.6 µCi/ml. At the end of this period, the cells were removed by low-speed centrifugation. Cellular debris was removed by centrifugation at 10,000 x g for 10 min at 2°. The supernatants were layered onto 7.5 ml of 15% sucrose in 10 mM Tris-HCl, pH 7.4:100 mM NaCl:1 mM EDTA, and the virions were pelleted by centrifugation at 82,500 x g for 2 hr at 2° in the 18-ml tubes of a Beckman SW 27 rotor. The pellets were extracted for 15 min at 4° in 2 ml Immune Buffer A (10 mM NaHPO\(_4\), pH 7.6:1 mM disodium EDTA:1% Triton X-100:0.5% sodium deoxycholate:0.1% sodium dodecyl sulfate). The extracts were centrifuged at 200,000 x g for 20 min at 2° in a Beckman type 65 rotor, and the pellets were discarded. The supernatants were subjected to secondary immune precipitation with purified antisera to Friend leukemia virus, and the precipitates were washed as described elsewhere (7). The washed immune precipitates were resuspended in 10 ml Immune Buffer A plus 1% bovine serum albumin. Goat antiserum to Rauscher leukemia virus p30 and gp70, were present in the medium from control and induced Friend 745 cells. Furthermore, the amounts of infective virus per cell section averaged approximately 2 for both types of cells, as based on analysis of 60 cells of each type. However, as described by Sato et al. (24), there is a broad range of numbers of budding virions per cell section. For example, Fig. 2 shows several small regions of control cell plasma membrane that contained several budding virions.

**Release of MuLV by Induced and Control Friend 745 Cells.** Friend et al. (10) reported that the MuLV’s released from these cells are low in leukemogenic activity although they are highly active as an immunizing agent. In agreement with that conclusion, only a very low number of infective MuLV’s were present in the cell culture medium (see Table 1). As shown in Table 1, similar amounts of infective virus were present in the medium from control and induced Friend 745 cells. Furthermore, the amounts of infective intracellular virus, as measured in the cell homogenate, were similar in the 2 cultures. Since infective virus probably represents only a minor fraction of the MuLV particles in Friend 745 cells, we measured the release of virions by an immunological method. The culture medium from cells that had been labeled with low specific activity L-\[^{3}H\]leucine for 20 hr was sedimented to concentrate particulate material that then was solubilized with detergents. The solubilized viral proteins were then immunoprecipitated with purified antiserum to Friend leukemia virus titer in the medium is normalized to the concentration of cells

<table>
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<tr>
<th>Cells used</th>
<th>Culture medium</th>
<th>Intracellular</th>
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<tbody>
<tr>
<td>Control</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Induced</td>
<td>41</td>
<td>67</td>
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**RESULTS**

**Electron Microscopy of Friend 745 Cells.** An electron microscopic examination of these cells was in accord with previous detailed studies (19, 24, 30, 33). As illustrated in Fig. 1, cells induced to synthesize hemoglobin for 96 hr are smaller than control cells, and their nuclei are relatively condensed. Induced cells have the same DNA content but only approximately 50% as much RNA and protein as do control cells (28). Furthermore, approximately 55% of the sections through induced cells contained cytoplasmic vesicular structures filled with C-type particles that were absent from control cells (see Fig. 1). In agreement with the report of Usery et al. (33) and in contrast with the report of Sato et al. (24), we observed approximately as many budding virions on the plasma membranes of control as induced cells. The numbers of budding virions per cell section averaged approximately 2 for both types of cells, as based on analysis of 60 cells of each type. However, as described by Sato et al. (24), there is a broad range of numbers of budding virions per cell section. For example, Fig. 2 shows several small regions of control cell plasma membrane that contained several budding virions.

**Table 1**

<table>
<thead>
<tr>
<th>Infective MuLV/10⁶ cells</th>
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<tr>
<td>Cells used</td>
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<td>Control</td>
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<td>Induced</td>
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made to Friend MuLV (see "Materials and Methods"), and the radioactivity was measured. As shown in Table 2, the control and induced cells released approximately the same amount of radioactive virus proteins. These results suggest that hematopoiesis does not cause enhanced release of MuLV particles.

**Intracellular p30 and gp70.** As measured by radioimmunoassays, control and induced Friend 745 cells contain approximately the same amounts of p30 and gp70 (Table 3).

**Synthesis and Processing of Viral Proteins in Induced and Control Friend 745 Cells.** For the determination of whether MuLV-specific protein synthesis is enhanced concurrently with hematopoiesis in Friend 745 cells, induced and control cells were pulse-labeled for 30 min with L-[\textsuperscript{35}S]methionine, and the virus-specific proteins were precipitated from cellular extracts with monospecific antiserum to p30 or gp70. As reported earlier, the induced cells are approximately one-half the size of the control cells, they contain fewer ribosomes and proteins, and they are less active in protein synthesis (24, 28). As shown in Table 4, the percentage of virus-specific protein synthesis is approximately 2-fold higher in the induced cells than in the control cells. This shows that synthesis of virus proteins is less inhibited by hematopoiesis than is total cellular protein synthesis. Nevertheless, since the overall rate of protein synthesis in the control cells is 4 to 5 times higher, the quantity of virus-specific protein synthesis per cell is approximately twice as high for the larger control cells as for the smaller induced cells.

**Table 2**  
*Release of virions by Friend erythroleukemia cells*

Control and induced Friend 745 cell cultures were labeled with L-[\textsuperscript{3}H]leucine, 28.6 \(\mu\text{Ci/ml}\), from 76 to 96 hr of the induction protocol. The cells were removed by centrifugation, and the culture medium was layered onto 15% sucrose in 10 mM Tris-HCl, pH 7.4-100 mM NaCl-1 mM EDTA. The virions were pelleted by centrifugation at 82,500 \(\times\) g for 2 hr at 2°. The pellets were extracted with 2 ml of Immune Buffer A and were centrifuged at 200,000 \(\times\) g for 20 min at 2°. The supernatants were made to Friend MuLV (see "Materials and Methods"), and the radioactivity was measured. As shown in Table 2, the control and induced cells released approximately the same amount of radioactive virus proteins. These results suggest that hematopoiesis does not cause enhanced release of MuLV particles.

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molecule of Friend virus migrates with an apparent molecular weight of 75,000. The protein with the molecular weight of 55,000 precipitated by antisera to gp70 is modified by glycosylation and has no known function (23). Chart 1 shows that all of these proteins are formed in a manner that is unaffected by hematopoiesis.

Comparison of Friend 745 Cells with Friend Leukemia Cells That Produce Infective Virus. To obtain information concerning the defectiveness of the virus produced by Friend 745 cells, we compared that cell line with an independently derived line of Friend leukemia cells (F4-6/K cells) that are approximately 5000 times more active in production of infective virus. The F4-6/K cells release both the lymphatic leukemia helper and the spleen focus-forming virions of the Friend virus complex (5, 19, 27). In a previous report, we showed that both cell lines contain similar amounts of virions as measured by p30 and reverse transcriptase measurements on particles precipitated from the culture medium with concanavalin A (27).

As shown in Table 5, these cell lines also synthesize similar amounts of virus-specific proteins that can be precipitated with antisera to p30 or gp70. Furthermore, as shown by the electrophoretic analysis in Chart 2, the sizes of the viral protein precursors and the synthesis patterns and processing pathways of these cells were indistinguishable.

**DISCUSSION**

Sato et al. (24) reported a striking phenomenon that has been confirmed in other laboratories (19, 30, 33), namely, that Friend cells induced to synthesize hemoglobin with dimethyl sulfoxide contain numerous cytoplasmic vesicles packed with virus that are absent from uninduced cells. Although we have confirmed this electron microscopic observation, our results indicate that virus-specific protein synthesis and processing and virion release from Friend 745 cells are similar in control and induced cells. Furthermore, the intracellular amounts of p30 and gp70 antigens are not increased by hematopoiesis (Table 3).

Our interpretation of these results is that MuLV-related gene expression is not appreciably affected by hematopoiesis in Friend 745 cells. Rather, the virus in the cytoplasmic vesicles of induced cells may occur simply because these cells contain the vesicular membranes that can serve as a site of virus assembly and intravesicular budding. The control cells, which lack these vesicles, contain a similar quantity of virion proteins that assemble and bud exclusively on the plasma membrane. The intravesicular virions probably are released from the cells only relatively slowly (Tables 1 and 2), and they probably contain only a small proportion of the total intracellular virion proteins (Table 3). The intravesicular virions probably become inactivated slowly in a manner similar to virions incubated in culture medium (27) because the induced cells do not accumulate infective virions (Table 1).

The synthesis and processing pathways for formation of Friend virus proteins (7), as described in "Results," differ somewhat from the pathways that have been described for Rauscher virus (1, 2, 18, 26, 34). In the latter case, the gag polyprotein with a molecular weight of approximately 75,000 is believed to be sequentially cleaved by proteolysis to produce the protein with the molecular weight of 65,000 and the smaller sized gag proteins. However, cells infected with Friend virus contain several glycosylated forms of the gag polyproteins (7). Another important difference is that Friend leukemia cells contain a glycoprotein with a molecular weight of approximately 55,000 that precipitates with antibody to gp70 (Charts 1 and 2. Ref. 23). We recently have obtained evidence that this glycoprotein is encoded by a previously undescribed virus component of the Friend virus complex.

The relationship of hematopoiesis to MuLV production by...
Dilution of concentrated cell suspensions with fresh media of infective virus are released when Friend cells are without inhibiting hematopoiesis (31), which suggests that cell lines that we have examined. Conversely, the hematopoietic process, at least in the Friend leukemia culture medium that contained dimethyl sulfoxide. How virus production is not required for hemoglobin synthesis. On the other hand, Dube et al. (5) have reported that virus production is not required for hemoglobin synthesis. C. C. Sherton et al.

Friend leukemia cells has remained somewhat unclear. Interferon blocks MuLV production in Ostertag’s cell lines without inhibiting hematopoiesis (31), which suggests that virus production is not required for hematopoiesis. On the other hand, Dube et al. (5) have reported that infective virus production is greatly stimulated by hematopoiesis in some of their cell lines. The virus was released in a wave 24 to 48 hr after dilution of the cells into fresh culture medium that contained dimethyl sulfoxide. However, we did control experiments that showed that similar waves of infective virus are released when Friend cells are diluted into medium that lacks dimethyl sulfoxide, that dilution of concentrated cell suspensions with fresh medium causes release of cells from a G_1 state of proliferative arrest, and that virus release occurs only in growing cells (28). A similar requirement for cell proliferation has been reported for exogenous and endogenous MuLV production by fibroblasts (9, 11, 16, 21, 22, 32). On the basis of these results, we suggest that MuLV synthesis is unaffected by the hematopoietic process, at least in the Friend leukemia cell lines that we have examined. Conversely, the hematopoietic process is also unaffected by MuLV synthesis (31).

REFERENCES


30. Sugano, H., Furusawa, M., and Ikawa, Y. Enhancement of Erythroucytic Maturation of Friend Virus-induced Leukemia Cells In

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Leukemia Virus Proteins in Friend Cells


Fig. 1. Electron micrographs of (A) control cells and (B) cells induced to synthesize hemoglobin for 96 hr. The cytoplasmic vesicular structures occur only in induced cells. × 8,500.
Fig. 2. Electron micrographs of control cells, showing portions of plasma membrane that appeared active in virus budding. A, × 47,500. B, 2 adjacent cells, both with budding virions. × 112,500.
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