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# Co-Suppression of Virus Production and Erythroid Differentiation in Friend Erythroleukemic Cell X Non-erythroid Mouse Cell Hybrids[1] (40411)

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Friend virus-infected erythroleukemia (FL) cells (1) provide a system for studying mechanisms controlling erythroid differentiation as well as virus multiplication. Enhanced erythroid differentiation, accompanied by increased hemoglobin synthesis, occurs when FL cells are grown in the presence of dimethyl sulfoxide (DMSO) (2). In addition, increased numbers of budding virus particles (3, 4) and higher virus titers in cultures undergoing DMSO-stimulated differentiation have been reported (5–8). Among the numerous hybrid clones which have been generated between the FL cells and other mouse cells, those involving a non-erythroid parent, such as L cells, teratocarcinoma cells, hepatoma cells and human fibroblasts (9–12, Skoultchi, Conscience and Ruddle, in preparation), showed complete extinction of the erythroid properties originally found in the parental FL cells. The extinction of erythroid properties in the clones selected for study had been previously documented by assaying for hemoglobin, globin mRNA, benzidine-positive (B+) cells, iron incorporation, acetylcholinesterase and carbonic anhydrase activity (9, 13). The constitutive level of these markers of erythroid differentiation was very low or undetectable as compared to that of untreated FL cells, and DMSO treatment did not cause an enhancement. It was therefore of interest to determine whether or not the viral functions were also extinguished in hybrid cells.

Virus synthesis was measured by assaying for the presence of reverse transcriptase (RT) activity in the medium of the clones grown with or without 1.0 or 1.5% DMSO. It was found that in most of the clones the ability to produce virus was suppressed and could not be activated by DMSO treatment. The virological properties of the hybrids were thus found to be similar to those of the non-erythroid parental cell.

*Materials and methods. Cell lines and tissue culture.* The origin and the method of selection of the hybrid clones used here have been described elsewhere (9, Skoultchi, Conscience and Ruddle, in preparation). Briefly, two FL lines were used as erythroid parents, both derived from clone 745 (1). FBU was a line of FL cells resistant to 30 μg/ml of 5-bromo-2′-deoxyuridine and was fully inducible for hemoglobin synthesis when exposed to DMSO, while FTG was resistant to 30 μg/ml thioguanine and had lost the capacity to increase hemoglobin production in response to DMSO. Except for their drug resistance and their inducibility, both lines shared the same general growth characteristics of the parental FL cells. Two subclones of mouse L cells were used as the non-erythroid parents: A9, azaguanine-resistant and lacking hypoxanthine–phosphoribosyltransferase activity, and LM(TK$^-$), bromodeoxyuridine-resistant and lacking thymidine kinase activity. Finally, Hepa 1a was an azaguanine-resistant mutant of a mouse hepatoma line. Fusion between FL and nonerythroid mouse cells was promoted with β-propiolactone-inactivated Sendai virus and the hybrid cells were selected in HAT medium. Many hybrid clones were characterized and all of them showed total extinction of erythroid properties, as described elsewhere (9, 13). Clones resulting from an FBU × A9 fusion are designated FA9; those from FTG × LM(TK$^-$) as LMFTG, and those from FBU × Hepa 1a as FHP.

The cells were grown in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY), supplemented with 10% fetal calf serum (Flow Labs, Rockville, MD), 100 units/ml penicillin, 100 μg/ml streptomycin and 3 m*M*

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glutamine. Hybrid lines were grown in HAT medium. Cells were seeded at $10^5$ cells/ml in 25 ml medium with or without DMSO. Concentrations of 1.0% or 1.5% were used, depending on the tolerance of the hybrid cells for this compound. They were grown for five to six generations to saturation (suspension cultures) or confluence (attached cultures) before harvesting. These culture conditions as well as the induction protocol have been described in detail elsewhere (9).

*Reverse transcriptase assay.* After clarification by centrifugation at 200*g*, the supernatant fluid from each culture was spun at 27,000*g* for 15 min in a Sorvall RC-2B centrifuge at 4°. The supernatant fluid was removed and the virus pelleted at 100,000*g* for 90 min in a Spinco 30 rotor at 4°, resuspended in 0.01 *M* Tris–HCl buffer (pH 7.6) containing 0.1M NaCl and 1 mM EDTA (NTE buffer), and stored at −96° before use. Exogenous viral RT activity was assayed, using the method of Mayer *et al.* (14) with modification.

The reaction mixture in a final volume of 50 μl contained: 50 m*M* Tris, pH 8.0, 30 m*M* NaCl, 2 m*M* dithiothreitol, 0.1 m*M* dATP, 0.8 m*M* $MnCl_2$, 0.02% (v/v) NP-40, 10 μg/ml poly $rA \cdot dT_{10}$ (Collaborative Res. Inc., Waltham, MA), 1 μCi[$^3$H]thymidine-5′-triphosphate (TTP) (57 Ci/mmole, Schwarz/Mann, Orangeburg, NY), 0.01 m*M* TTP and 25 μl of concentrated virus suspension.

The mixture was incubated at 37° for 1 hr, stopped with 10 μl of 0.2 *M* EDTA and chilled in ice. A volume of 50 μl was spotted on a 25 mm disc of DE-81 filter (Whatman, Inc., Clifton, NJ) which was washed six times with 5% $Na_2HPO_4$ (4 min per wash), and twice with water, 95% ethanol and ethyl ether (15). Filters were immersed in a toluene-based scintillation fluid and counted. RT activity was expressed as [$^3$H]-TMP incorporation per mg cellular protein (2000 cpm as 1 pmole). All assays were carried out in duplicate reaction mixtures with zero time subtracted.

*Protein measurement.* The cell pellets were washed with phosphate buffered saline, lysed with 1% NP-40 and sonicated. Soluble protein in the lysate was determined according to Lowry *et al.* (16), using crystallized bovine serum albumin (Sigma) as the standard.

*Results.* The level of virus released by the untreated parental cultures is shown in Tables I and II. In control parental FL cell cultures (FBU and FTG), whether or not they were inducible for hemoglobin synthesis, there were high levels of virus production, whereas in the L cell lines (A9, LM(TK$^-$)) and the hepatoma cells (Hepa 1a), the level of RT activity was not significant. An arbitrary setting of 20,000 cpm/mg protein was considered a significant level of virus production since it represented about 1,000 cpm (0.5 pmole) of [$^3$H]TMP incorporation per assay. When FL cells were grown in the presence of 1.5% DMSO, there was a twofold increase of virus over that of the control. DMSO not only failed to stimulate virus production in A9 (which is supposedly virus-free), LM(TK$^-$) and Hepa 1a cells, but appeared to cause a decrease (Tables I and II).

Among 16 FA9 clones, 4 LMFTG clones (Table I) and 17 FHP clones (Table II) tested for RT activity, most of them showed little or no virus production. A few clones (FA 2a, 15a in Table I and FHP 4a, 9c, 10a, 11b, 16a and 19c in Table II) showed slightly higher RT activity as compared to that of the A9 or Hepa 1a parent, but all were less than 30% of that of the FL cell parent. Treatment with 1.5% DMSO did not enhance virus production in any of the 16 FA9 clones. Similar results were obtained with most FHP hybrids (1.0% DMSO) and LMFTG hybrids (1.5% DMSO). However, significant stimulation of virus release occurred in two hybrids, FHP 1a and LMFTG 15a. Although these two clones grew poorly in the presence of DMSO, the stimulation appears to be significant. Treated clones FHP 1c and 2b also showed slightly increased RT activity.

*Discussion.* The total extinction of the erythroid characteristics expressed in the FL parent cell from FL hybrid clones had been demonstrated previously (9, 13, Skoultchi *et al.*, in preparation). Assays for hemoglobin synthesis, globin mRNA production, B+ cells, stimulation of iron incorporation, acetyl-cholinesterase and carbonic anhydrase were negative (9, 13). Although the mechanism which caused the extinction was not clear, the data indicated that the genome of the nonerythroid parent might be actively inhibiting the expression of the erythroid

TABLE I. REVERSE TRANSCRIPTASE ACTIVITY IN FL CELL X MOUSE L CELL HYBRID CULTURE FLUID.

| Cell | DMSO | [$^3$H]TMP incorp (cpm × $10^{-3}$/mg cell protein) | Cell | DMSO | [$^3$H]TMP incorp (cpm × $10^{-3}$/mg cell protein) |
|---|---|---|---|---|---|
| FBU | − | 139.0 | FTG | − | 186.5 |
| | + | 328.4 | | + | 384.1 |
| A9 | − | 7.0 | LM(TK$^-$) | − | 19.1 |
| | + | 1.6 | | + | 0.0 |
| (FBU × A9 hybrid) | | | FTG × LM(TK$^-$) hybrid | | |
| FA9 2a | − | 30.1 | LMFTG 7a | − | 4.5 |
| | + | 3.8 | | + | 1.9 |
| 3a | − | 4.0 | 15a | − | 3.6 |
| | + | 0.2 | | + | 41.0 |
| 3b | − | 6.0 | 18a | − | 5.9 |
| | + | 7.9 | | + | 4.5 |
| 4a | − | 1.2 | 20a | − | 7.2 |
| | + | 1.8 | | + | 5.1 |
| 10a | − | 1.9 | | | |
| | + | 0.4 | | | |
| 11a | − | 5.0 | | | |
| | + | 1.5 | | | |
| 11b | − | 1.6 | | | |
| | + | 0.5 | | | |
| 12a | − | 1.4 | | | |
| | + | 1.4 | | | |
| 13a | − | 2.3 | | | |
| | + | 0.4 | | | |
| 13b | − | 8.1 | | | |
| | + | 0.0 | | | |
| 14a | − | 8.4 | | | |
| | + | 0.4 | | | |
| 15a | − | 16.3 | | | |
| | + | 0.5 | | | |
| 15b | − | 4.0 | | | |
| | + | 0.3 | | | |
| 16a | − | 3.5 | | | |
| | + | 0.2 | | | |
| 17a | − | 2.6 | | | |
| | + | 0.7 | | | |
| 20b | − | 1.2 | | | |
| | + | 0.1 | | | |

genes at a pretranslational level (9).

These hybrids have now been further characterized on the basis of their virological properties. The extinction of viral gene expression following cell hybridizaiton was found to parallel that of erythroid expression. All the hybrid clones assayed possessed the properties of the non-virus-producing parent cell. The constitutive levels of virus released by the hybrid clones was reduced to very low or undetectable values as compared to those of the FL parent. Treatment of the hybrid cell cultures with DMSO did not enhance virus production. Thus, in the hybrid cell cultures, hemoglobin and virus production appeared to be co-suppressed. On the other hand, in spite of indirect evidence (9), the possibility that the co-inactivation of both functions in our hybrids was due to a physical loss of specific genes involved with the regulation of erythroid differentiation and virus production cannot be excluded.

*Summary.* The production of virus in somatic cell hybrids between Friend erythroleukemia cells and nonerythroid mouse L cells or hepatoma cells was examined. Virus syn-

TABLE II. REVERSE TRANSCRIPTASE ACTIVITY IN FL CELL X MOUSE HEPATOMA CELL HYBRID CULTURE FLUID.

| Cell | DMSO | [$^3$H]TMP incorp (cpm × $10^{-3}$/mg cell protein) |
|---|---|---|
| FBU | − | 139.0 |
| | + | 328.4 |
| Hepa 1a | − | 0.5 |
| | + | 0.2 |
| (FBU × Hepa 1a hybrid) | | |
| FHP 1a | − | 1.4 |
| | + | 55.5 |
| 1c | − | 2.6 |
| | + | 4.1 |
| 2b | − | 0.5 |
| | + | 1.4 |
| 3a | − | 5.2 |
| | + | 0.0 |
| 4a | − | 19.9 |
| | + | 5.1 |
| 4b | − | 2.8 |
| | + | 2.5 |
| 7b | − | 0.0 |
| | + | 0.0 |
| 8a | − | 4.1 |
| | + | 1.8 |
| 9a | − | 4.9 |
| | + | 0.0 |
| 9b | − | 4.9 |
| | + | 0.0 |
| 9c | − | 51.6 |
| | + | 15.4 |
| 10a | − | 3.6 |
| | + | 0.8 |
| 11b | − | 10.6 |
| | + | 0.5 |
| 13b | − | 2.7 |
| | + | 0.0 |
| 15a | − | 8.3 |
| | + | 14.9 |
| 16a | − | 37.5 |
| | + | 3.9 |
| 19c | − | 16.4 |
| | + | 4.0 |

thesis was determined by assaying particle-associated reverse transcriptase activity in the culture fluid. Virus production was found to be suppressed in the 37 hybrid clones previously shown to have complete extinction of erythroid differentiation. Treatment of the cultures with DMSO, which enhances virus release in the parental erythroleukemic cells, did not result in recovery of the ability of the hybrids to produce virus.


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