# Possible Role of the Friend Virus Life Cycle in Differentiating Friend Leukemia Cells Treated with Interferon

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## A. Introduction

Friend erythroleukemia cells (FLC) are proerythroblasts chronically infected with the Friend virus complex (FLV, composed by LLV and SFFV). They undergo erythroid differentiation accompanied by synthesis of heme and hemoglobin (Hb), accumulation of globin mRNA and erythrocyte-specific membrane changes upon treatment with DMSO and other polar solvents [6].

Data collected in the past 2–3 years have convincingly shown widespread pleiotropic effects of interferon (IF). As reviewed elsewhere [1], inhibitory as well as stimulatory effects of IF on various types of cells have been reported. As for the antiviral activity of IF, it has been shown that in cell chronically infected wi RNA tumor viruses, IF treatment suppresses extracellular virus production, but does not abolish intracellular virus antigens expression [4]. We have been studying the action of DMSO and IF upon growth and differentiation of FLC, on one hand, and the effect of IF on the FLV genome expression, on the other hand.

## B. Control of FLC Differentiation by Interferon

In previous papers we have described in detail the effects exerted by high doses of IF on FLC growth, cell cycle and erythroid differentiation [9,11,12].

FLC growth is inhibited, in a dose-dependent fashion, by the administration of IF doses higher than 500 U/ml. Cell cycle parameters also change with respect to $G_1$ and $G_2$ phases, that are almost doubled in length. Removal of IF results in a prompt resumption of growth potential and normal cycle parameters. Similar data were obtained when DMSO-stimulated FLC were treated with IF. Under conditions allowing at least 2–3 cell doublings in the presence of DMSO, Hb synthesis was reduced by 90%, but globin mRNA only by 30%, in FLC treated with DMSO + IF. It is apparent that the observed transcriptional effect of IF, although a novel one for a "cellular" mRNA, cannot fully explain the magnitude of the translational inhibition. In addition, these globin mRNAs (from DMSO- and from DMSO + IF-treated FLC) are indistinguishable from one another for a) base sequence, as deter-

mined by Tm, b) size, analyzed by 99% formamide, 4,5% polyacrylamide gel electrophoresis, and c) ability to direct the synthesis of globin-size materials in homologous ($S_{30}$ lysates) and heterologous (wheat-germ) cell-free protein-synthesizing systems. Nonetheless, DMSO + IF-treated FLC do not synthesize appreciable amounts of globin $\alpha$ and $\beta$ chains, although they do apparently produce significant amounts of a protein(s), more cationic than $\beta$ chain, immunoprecipitable with a monospecific globin antiserum.

IF doses lower than 100 U/ml do induce, instead, a substantial increase of Hb-producing cells in DMSO-stimulated cultures [1]; data in Table 1 demonstrate that this effect is mediated by increase of globin mRNA levels. Preliminary results, moreover, indicate that the administration of low doses of IF stimulates erythroid differentiation of FLC *per se*, i.e. in the absence of any induction by DMSO; increased amounts of globin mRNA have been observed in IF-treated FLC as compared to untreated FLC (Table 1).

**Table 1.** Erythroid differentiation and viral gene expression, on day 3 of culture, of FLC given low dosages (25 U/ml) of IF on day 0

| Treatment | Benzidine-positive cells (%) | Globin mRNA copies/cell | LLV-specific RNAs (% of cytoplasmic RNAs) |
|---|---|---|---|
| none | 1 | 157 | 0,022 |
| + IF | 12 | 556 | 0,015 |
| + DMSO | 41,7 | 1641 | 0,044 |
| + DMSO + IF | 50,2 | 2434 | 0,009 |

## C. Life Cycle of FLV in FLC Treated with Interferon

The extracellular virus release is impaired by IF [4], whereas the synthesis of FLV-specific proteins is not blocked, so that intracytoplasmic accumulation of such proteins occurs [3].

We have analyzed cytoplasmic RNAs extracted from IF-treated FLC, with/without simultaneous treatment with DMSO, by molecular hybridization with cDNA probe complementary to the LLV component of FLV. Results are shown in Table 1: while in DMSO-treated FLC there is a significant increase of viral RNAs, IF treatment alone barely reduces the virus-specific cytoplasmic RNA content. The combined treatment, DMSO + IF, instead, significantly decreases the viral RNA concentration as compared both to untreated and to DMSO-treated FLC.

Possible explanation of these results could be: either IF operates at both an early (transcription) and a late (assembly) step of virus cycle, or, alternatively, the intracellular levels of virus RNAs are controlled by the accumulated viral proteins in a "feedback-type" inhibition.

## D. Discussion

In conclusion, we have reported here a set of data, apparently unrelated to one another, namely the peculiar inhibitory action of IF on the life cycle of FLV, and the "pendulum" type of effect on FLC differentiation. It seems to us, however, that there is a possibility of giving a unifying interpretation.

I. Recent evidence [5,14], contrary to what has been assumed so far, suggests that the LLV component of the FLV complex is able to induce erythroid leukemias on its own in susceptible newborn mice. The question raises as to what role SFFV is playing in the biology of the FLV system, particularly in the as yet unknown mechanism(s) triggering on erythroid differentiation in vitro.

One possible answer could be the following one: SFFV induces high levels of polycythemia in susceptible mice. Whether or not the SFFV interaction(s) by itself with its target cells results also in leukemic transformation is still not clear. It seems reasonable, however, to assume that its ability to induce polycythemia in vivo may play a critical role in providing the lymphatic leukemia virus component (LLV) with a much larger subpopulation of "erythroid-committed" precursor cells. These cells would then become available for the transforming event. Such transformation, therefore, would be the result of the interaction of these cells with LLV. An erythroleukemia would then ensue as a consequence of a combined and sequential action of the two viruses.

This is in agreement with the finding that CFU-E's from mice injected with the polycythemic strain of FLV (FLVp, composed by LLV and SFFV) are a mixture of erythropoietin-dependent and erythropoietin-independent cells, whereas CFU's from mice given the anemic strain (FLAa, only LLV composed) are all erythropoietin-dependent (Peschle and Rossi, unpublished data). According to the above mentioned hypothesis, the conversion of the CFU-E's from erythropoietin-dependency to erythropoietin-independency would be accomplished by the SFFV component. This hypothesis would also explain why the SFFV-deprived preparations of FLVp induce "late" lymphatic leukemias, whereas it does not account for the fact that the anemic strain FLVa, which is free of SFFV from the origin, is also able to cause erythroleukemias, and only these, in vivo. Minor, and as yet undetected, differences between the two strains of FLV-LLV may be responsible for this apparent discrepancy.

II. In the in vitro system (the Friend cells), treatment with DMSO or with other inducers causes a pronounced erythroid differentiation accompanied by increased production of FLV complex (LLV and SFFV). It is very interesting to compare the kinetics of the increased production of SFFV with that of LLV in the framework of the "induced" erythroid differentiation. The stimulation of LLV by DMSO is quantitatively small (2–5 fold) and occurs later (3–4 days after induction) [2,10]. Such LLV stimulation, therefore, follows the erythroid differentiation, whose early parameters (spectrin, glycophorin, nonhistone chromatin changes) have been detected much earlier [6,8,13]. The SFFV increase, instead, is a very early and much more pro-

nounced phenomenon [10], and it seems to occur prior to the appearance of the early markers of erythroid differentiation, suggesting that it might be the real "trigger" of differentiation.

III. This hypothesis would also help in understanding the IF "pendulum" type of action on erythroid differentiation. It could be due, of course, to some inherent properties of the IF molecules, but one may also offer an alternative explanation, based on interactions between IF and the two components of the FLV complex. The relative sensitivity of SFFV and LLV to IF may be different. Differences in the susceptibility of viruses to IF have been reported [7]. We postulate that LLV is sensitive even at low doses of IF, whereas SFFV is sensitive only to high doses (above 500 U/ml). At low doses only LLV would be inhibited with a relative enrichment of SFFV, and consequently with some enhancement of erythroid differentiation. At high doses, instead, SFFV would also be blocked, with a consequent inhibition of erythroid differentiation.

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