Modulation of Hemoglobin Synthesis in K-562(S) Cells Treated With Interferons

By Livia Cioè, Pacifico Meo, Vincenzo Sorrentino, and Giovanni B. Rossi

Treatment of K-562(S) cells with human interferons (HuIFN) α, β, or γ results in a modulation of hemoglobin (Hb) production. When K-562(S) cells, “induced” with butyric acid or hemin, are given low dosages of all three types of IFN, the percent benzidine-positive cells doubles. Treatment with low doses of IFN causes the acceleration and increased production of those Hb types (as shown by Cellogel analysis) that are already synthesized either in untreated or in “induced” cultures. These results were confirmed by using pure HuIFN-β, and were abrogated, for HuIFN-α, in presence of its specific antiserum. In contrast, cultures of K-562 cells treated with either inducer and given more than 10^4 IU/ml of α- or β-IFN show a dose-dependent decrease of hemoglobinization in the absence of cell death. The inhibitory effect was reversible upon removal of IFN and culture reseding in IFN- and inducer-free medium. The significance of both sets of data is strengthened by the pronounced heterogeneity of K-562S cells with respect to their sensitivity to IFN treatment as evaluated by the establishment of the antiviral state. Apparently, one cell out of two is sensitive to IFN, which suggests that the magnitude of IFN effects described here may be larger than it appears to be from the data taken at face value.

In this article we describe the effects of treatment of K-562S cells with three types of human IFN both in the presence and the absence of butyric acid or hemin. The administration of low doses of any IFN type results in acceleration and enhancement of K-562S hemoglobinization, whereas cultures given more than 10,000 International Units (IU)/ml of human interferon (HuIFN)-α or -β show a marked decrease of percent benzidine-positive cells. Hbs detected by Cellogel analysis of lysates from IFN-treated K-562 cells are always of the same type synthesized in the corresponding control population. Effects produced by low HuIFN-β doses have been confirmed by using pure HuIFN-β preparations, and those of low HuIFN-α doses have been abrogated by treatment of the cultures with α-IFN previously incubated with its specific antiserum.

MATERIALS AND METHODS

Cells

K-562S1 and Friend leukemic cells were grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS) in 5% CO2 humidified atmosphere. K-562S cells were seeded at 2 × 10^5/ml in medium previously conditioned with hemin (Sigma Chemical Co., St. Louis, MO) or butyric acid (But.A., Fluka, Buchs, Switzerland). Four days later, cells were stained with benzidine44,45 to detect Hb production in whole cells. Hb amounts were also assessed in cell pellets washed twice with phosphate-buffered saline (PBS), resuspended in an equal volume of distilled water, lysed by 3 cycles of freezing-thawing in liquid nitrogen, centrifuged once at 2,000 rpm for 10 min, and then at 15,000 rpm for 45 min. The visible absorbance spectrum of the supernatants was determined to identify the material as Hb,13 which was then quantitated at 414 nm, assuming that an absorbance of 1.0 at 414 nm is equivalent to 0.0945 mg/ml.18

Hb Separation

Total cell lysates from 10^6 cells, obtained as above, were electrophoresed on Cellogel (Chemotron, Milan, Italy) in borate buffer pH 8.67 at 5 mA for 2 hr. Hb bands were visualized by benzidine staining using O-Dianisidine.44,45

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Interferons

Mouse fibroblast IFN was prepared by infecting aged mouse L29 fibroblasts with 1–10 hemagglutinating units/10^6 cells of Newcastle disease virus. The virus was removed after 1 hr of adsorption, monolayers washed with PBS and incubated with medium containing 3% fetal calf serum for 18 hr. Clarified supernatants were brought to pH 2.0 with 6N HCl, kept at 4°C for 5 days, neutralized to pH 7.0, and stored at −80°C. The specific activities of murine interferone (MuIFN) preparations averaged 5 x 10^5 IU/mg protein.

Human leukocyte IFN (HuIFN-α) (courtesy of Dr. K. Cantell, Central Public Health Laboratory, Helsinki, Finland), had a specific activity of −10^6 IU/mg protein. Anti-HuIFN-α sheep serum (courtesy of Dr. K. Cantell) had a titer of 1:5 x 10^3 and was used at a 1:50 dilution. Control serum from unimmunized sheep (courtesy of Dr. Paola Verani Borgucci, Istituto Superiore di Sanità, Rome, Italy) was also used at 1:50 dilution.

Human crude type-II IFN (HuIFN-γ) (courtesy of Dr. F. Dianzani, Institute of Virology, University of Rome) was obtained from buffy-coat Ficoll-derived human lymphocytes treated with 10 U/10^6 cells/ml of galactose oxidase.

Human fibroblast IFN (HuIFN-β) was derived from human amniotic cells (UAC, courtesy of Dr. Cantell) induced for 1 hr with 10 hemagglutinating units/10^6 cells of Newcastle disease virus, and then added with fresh medium ± 2% newborn calf serum. Supernats from induced cells were harvested 20 hr later, clarified, brought to pH 2 with 6N HCl, kept at 4°C for 5 days, neutralized with 6N NaOH, and stored at −70°C. Studies of its activity after antiserum neutralization indicate that UAC-derived IFN consists only of IFN-β.19 Following partial purification on blue Sepharose chromatography column, its specific activity was −10^7 IU/mg protein. Pure preparations of HuIFN-β (gift of Dr. E. Knight, DuPont Corp., Wilmington, DE) had a specific activity of 5 x 10^6 IU/mg protein.

Murine and human IFN preparations were titrated on murine L29 cells, and respectively, on human primary embryo fibroblasts ESM (courtesy of Dr. A. Billau, Rega Institute, Leuven, Belgium) in a microtiter assay based on the inhibition of the cytopathic effect produced by infection with vesicular stomatitis virus (VSV). Every assay was carried out in the presence of an internal IFN standard preparation calibrated against NIH standards (Murine IFN α + β, code G002-904-511, HuIFN-β, code G023-902-527, and HuIFN-α, code G023-901-527). HuIFN-α and -β amounts given throughout this paper are expressed in international units. Human type-II IFN was titrated in the same microtiter assay. In the absence of an international standard preparation, its titers are given in laboratory units.

Virus

VSV stocks were prepared on mouse L29 fibroblasts and titrated by standard plaque assay. Virus titers averaged 10^6 plaque-forming units (PFU)/ml.

Infectious Centers Assay

A quantity of 10^7 K-562S and FLC cells, exposed to graded amounts of IFN for 24 hr, were washed free of IFN and infected with 5 PFU/cell VSV. After 1-hr adsorption, cell pellets washed free of unadsorbed virus by 3 cycles of low-speed centrifugation and resuspended in 0.1 ml undiluted anti-VSV rabbit serum (neutralization titer against 100 PFU of VSV = 1:5,120) were incubated at 37°C for 15 min. Then, 0.9 ml medium was added and pellets were further kept at 37°C and at +4°C for 15 min each time. After 3 cycles of low-speed centrifugation to remove excess antiserum, quadruplicate 0.1-ml samples of log_10 cell dilutions were plated on to confluent monolayers of indicator L29 cells in 35-mm plastic dishes. Plated cells were gently mixed with 0.4 ml 1% Bactoagar solution in 3% FCS phenol red-free medium to obtain a thin agar overlay. After solidification, 1.5 ml of the same agar solution was added and plates were incubated at 37°C in a 5% CO2 humidified atmosphere for 48 hr. Plaques resulting from VSV shed by individual plated cells were scored by vital staining (1:5,000 neutral red in saline) of cell monolayers.

RESULTS

IFN Sensitivity of K-562 Cells

Since a number of human20 and mouse21,22 cell lines have been found to be spontaneously resistant to the homologous IFN, the sensitivity of K-562S cells to the antiviral state induced by α- and β-type of human IFN was assessed.

Cells of either line were exposed to graded IFN amounts for 24 hr, then cell pellets were infected with 5 PFU/cell VSV. After 1-hr adsorption at 37°C, unadsorbed virus was removed and cultures incubated at 37°C for 8 hr in fresh RPMI medium. VSV yields in the supernatants were measured by plaque assay. As shown in Fig. 1, where data on IFN sensitivity of murine FLC are also shown for comparison, it appears that K-562S cells display a different susceptibility pattern to either IFN type than that exhibited by FLC. IFN dose–response curve of FLC starts to fall from 220 IU/ml downward, so that VSV yields from cells exposed to 800 IU/ml were reduced by 2 logs. As IFN units are established in assays carried out on mouse L29 cells, it appears that FLC are markedly less susceptible to IFN than L29 cells. IFN dose–response curves of K-562 cells are completely different, as they exhibit a hyperbolic pattern for both α- and β-IFN. Almost a log reduction of VSV yields is observed in cells given up to 100 IU/ml of IFN. When, however, 50-fold higher amounts of IFN are administered, no further reduction of virus production is detected. These data are compatible with the assumption that K-562 cells are heterogeneous with respect to susceptibility to the IFN-induced antiviral state, i.e., a fraction of K-562 cells exhibit a clear-cut IFN sensitivity (almost 1-log reduction of VSV yield after exposure to 100 IU/ml of IFN), whereas the remainder are apparently resistant to IFN, as no further reduction of virus yields is observed in face of 50-fold higher IFN amounts employed.

Both FLC and K-562S cells were therefore assayed for their individual capacity to sustain VSV replication following 24-hr treatment with graded doses of fibroblastic and leukocyte IFN, respectively, through a standard “infectious centers” assay. As shown in Fig. 1, untreated FLC exhibit an “infectious centers” (i.c.) plating efficiency of about 25%, which abruptly falls to 2.0% and 1.5% when cells were pretreated with 10 and

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30 IU/ml of IFN, respectively. These data indicate that FLC are rather homogeneous in their susceptibility to the induction of the antiviral state by IFN, which fits well with the IFN dose–response pattern of FLC as measured by VSV yield reduction (see above). On the other hand, untreated K-562S cells, which show a lower (6%) i.e. plating efficiency, exhibit a fully different pattern of α-IFN sensitivity. In fact, i.e. plating efficiency falls by 50% (3.2%) in cells previously exposed to 100 IU/ml of IFN, but declines poorly

![Graph](attachment:image1.png)

**Fig. 1.** Comparison of IFN sensitivity between K-562 and FLC cells. 10 x 10⁶ cells (either K-562 or FLC) were exposed to the indicated amounts of IFN for 24 hr. Cell pellets were washed free of IFN and infected with 5 PFU/cell vesicular stomatitis virus. After 1-hr adsorption at 37°C, unadsorbed virus was removed and cultures incubated at 37°C in fresh medium for 8 hr. VSV yields in the supernatants were measured by plaque assay on L₉₂9 murine cells. VSV yields are expressed as log PFU/ml. Replicate VSV-infected cell pellets were assayed in an “infectious centers” test, as in Materials and Methods. (A) VSV yields of K-562 treated with murine fibroblast IFN; (B) VSV yields of K-562 treated with HuIFN-α; (C) VSV yields of K-562 treated with HuIFN-β; (D) infectious centers/10⁵ FLC treated with murine IFN; (E) infectious centers/10⁵ K-562S treated with HuIFN-α.

![Graph](attachment:image2.png)

**Fig. 2.** Dose–response curves of butyric acid and hemin induction of K-562 erythroid differentiation. K-562 cells were seeded at 2 x 10⁷/ml in the presence of the indicated concentrations of either butyric acid (A) or hemin (B). Four days later, percent benzidine-positive cells was determined as described in Methods.
thereafter in cells exposed to up to 10,000 IU/ml of IFN. These data apparently confirm the heterogeneity of K-562S cells with respect to IFN sensitivity and suggest that roughly 1 cell out of 2 is sensitive.

Effects of Administration of Low Doses of α-, β-, or γ-Types of IFN

K-562 cell cultures, with or without butyric acid or hemin, were exposed to doses of IFN up to 5,000 IU/ml for 4 days, cells were counted in a hemocytometer, and their hemoglobinization assessed by determining the percent benzidine-positive (B+) cells or Hb content/cell according to Kabat et al. Pilot experiments showed that treatment with α-IFN resulted in unchanged growth curves but in increased B+ values in untreated as well as in induced cultures. Induction of K-562 cells with optimal doses of hemin (5 × 10⁻⁶ M) or butyric acid (1.2 mM) saturates the ability of these cells to express erythroid markers. It was therefore necessary to establish suboptimal doses of either inducer to amplify the differentiation-enhancing effects of IFN over those already exerted by the inducers themselves. In Fig. 2, dose–response curves for the two inducers under study are shown. Concentrations of 0.1 mM and 5 × 10⁻⁶ M were selected, respectively, for butyric acid and hemin and used in all experiments dealing with the effects of low doses of IFN.

Data obtained from K-562 cultures either untreated or given butyric acid or hemin and conditioned with graded doses of either α- or β-type of IFN are shown in Fig. 3. All dose–response curves are bell-shaped, peak at 500 IU/ml, and are closely superimposable with respect to the type of IFN employed. Similarly shaped dose–response curves were also obtained from K-562 cultures exposed to γ-type IFN (data not shown for the sake of clarity). As already observed for IFN-treated FLC not given any inducer, the administration of IFN to uninduced K-562 cells results in a limited but reproducible induction of B+ cells over baseline values.

The kinetics of the effects of α-IFN is shown in Fig. 4. Replicate K-562 cultures, seeded in the absence of inducers (A), with butyric acid (B), or with hemin (C) and given 500 IU/ml of α-IFN on day zero, were sampled at 24-hr intervals and percent B+ cells as well as Hb pg/cell were determined. As already reported, the kinetics of induction of Hb by hemin or butyric acid are different, the former being markedly faster. Cells induced by hemin also contain higher amounts of Hb/cell. IFN administration to induced cultures does not modify the respective kinetic patterns and apparently accelerates the expression of Hb genes in K-562 cells from day 2 onward. The same is true for uninduced K-562 cells treated with IFN. α-IFN was also added 1, 2, or 3 days after culture seeding ± inducers. Enhancement of Hb production is observed only when IFN addition is delayed 1 day (data not shown).

Specificity of IFN Effects

Data shown in Fig. 3 have been fully confirmed by using pure HuIFN-β preparations (Table 1). Further, as no pure HuIFN-α was available to us, the specificity of the effects induced by low dosages of this IFN type has been tested by using an anti-HuIFN-α sheep serum. The indicated α-IFN doses shown in Fig. 3 have been incubated for 1 hr at 37°C with either fresh medium or IFN antiserum or a control serum from nonimmunized sheep. Both sera were utilized at 1:50 final dilution. The mixtures were then added to test cultures, which were assayed for percent B+ cells 4 days later. As shown in Fig. 3, preincubation of
HuIFN-α with its specific antiserum fully abrogates IFN-induced enhancement of either spontaneous or butyric-acid-induced erythroid differentiation. The addition of anti-HuIFN-α serum or of the control serum to uninduced or induced cells in the absence of IFN results in a marginal decrease of percent B+ cell values, which is not statistically significant.

Electrophoretic Analysis of Hbs Produced in K-562S Cells Upon Induction With Butyric Acid or Hemin and Treated with α- or β-IFN

K-562 cells seeded in the presence of either inducer and given 500 IU/ml of α- or β-IFN were incubated for 4 days, lysed in equal volumes of distilled water, and supernatants were run on a Cellogel to separate Hbs. Gels were stained with 0-dianisidine to visualize Hb bands (Fig. 5). As already reported,1,5 treatment with optimal doses of butyric acid or hemin in untreated (empty symbols) or treated with suboptimal doses of either inducer (butyric acid 0.1 mM, hemin 5 × 10⁻⁶ M), only bands corresponding to Hbs Gowen I and Portland (α₂γ₂) are clearly visible. Yet, not even faint bands corresponding to these Hbs were visible (data not shown), indicating that treatment with suboptimal doses of the inducers causes the expression of only two types of Hb. When 500 IU/ml of α-IFN was added to cultures either uninduced (slot f) or treated with suboptimal (slots g and h) or optimal (slots i and l) inducer doses, Hb patterns were identical to those observed in the corresponding control populations, i.e., IFN treatment results only in “enhancing” the expression of those Hb types already expressed in IFN-free cultures. Essentially similar Hb patterns (Fig. 6) are obtained when 500 IU/ml of partially purified HuIFN-β (specific activity ~10⁷ IU/mg protein), were administered to untreated (slot d) or “induced” (slots e and f) cultures. Slots a, b show Hb patterns from K-562 cells induced with hemin or butyric acid (optimal doses) to provide the Hb markers. The specificity of this IFN effect was tested by using a pure (specific activity 5 × 10⁶ IU/mg protein) β-IFN preparation. Data in Fig. 7 are identical to those in IFN-free cultures taken as 100.

Table 1. Effects of Low Doses of Pure HuIFN-β on K-562 (S) Erythroid Differentiation

<table>
<thead>
<tr>
<th>Pure β-IFN (IU/ml)</th>
<th>None</th>
<th>Butyric Acid (0.1 mM)</th>
<th>Hemin (5 × 10⁻⁶ M)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>7 (100)†</td>
<td>23 (100)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>100</td>
<td>6 (92)</td>
<td>22 (92)</td>
<td>14 (92)</td>
</tr>
<tr>
<td>300</td>
<td>15 (210)</td>
<td>36 (166)</td>
<td>16 (102)</td>
</tr>
<tr>
<td>500</td>
<td>20 (280)</td>
<td>35 (158)</td>
<td>27 (180)</td>
</tr>
<tr>
<td>1,000</td>
<td>21 (300)</td>
<td>33 (140)</td>
<td>25 (166)</td>
</tr>
<tr>
<td>2,000</td>
<td>26 (370)</td>
<td>30 (120)</td>
<td>20 (133)</td>
</tr>
</tbody>
</table>

*As pure HuIFN-β was available in limited amounts, the data are from a single experiment run on duplicate samples.
† Numbers in parentheses represent the percent variation of B+ values as compared to those in IFN-free cultures taken as 100.
Fig. 5. Hemoglobin patterns of K-562 cells treated with 500 IU/ml of HuIFN-α. K-562 cells, seeded at 2 x 10⁶/ml in the absence or in the presence of appropriate doses of the inducers, were treated with 500 IU/ml of HuIFN-α. Four days later, 10⁶ cells were pelleted, washed with PBS, and lysed as in Materials and Methods. Supernatants were run on Cellogel, and gels were stained as described in Materials and Methods. Markers for HbF and A were obtained by parallel electrophoresis of cord blood. The identification of the other Hbs was based on their relative migration at alkaline pH, as previously described. 2 Slots: (a) untreated K-562 cells; (b) cells induced with 1.2 mM butyric acid; (c) cells induced with 0.1 mM butyric acid; (d) cultures induced with 5 x 10⁻⁶ M hemin; (e) cells induced with 5 x 10⁻⁶ M hemin; (f) uninduced K-562; (g) 0.1 mM butyric-acid-treated cells; (h) 5 x 10⁻⁶ M hemin-treated cells; (i) 1.2 mM butyric-acid-induced cultures; (j) 5 x 10⁻⁶ M hemin-induced cultures. All cultures in slots f–l were also given 500 IU/ml HuINF-α.

Effect of High Doses of α- or β-IFN on K-562
“Induced” Erythroid Differentiation

In keeping with data obtained with murine FLC given IFN, 13 the addition of more than 10,000 IU/ml of α-IFN to cultures seeded in the presence of optimal inducer dosages causes a dose-dependent inhibition of cell hemoglobinization (Table 2). Growth of IFN-treated cells is slowed down to 50% of control values from IFN doses of 40,000 IU/ml upward. Cell mortality, as measured by dye-exclusion test, is unremarkable and always below 8%. Replicate cultures of those

Fig. 6. Hemoglobin patterns of K-562 after treatment with 500 IU/ml partially purified HuIFN-β. Cell pellets and lysates were prepared as described in legend to Fig. 5, but instead of HuIFN-α, 500 IU/ml of partially purified HuIFN-β (specific activity ~10⁵ IU/mg protein) were used. Supernatants were run on Cellogel and stained as above described. Slots: (a) 1.2 mM butyric-acid-treated K-562 cells; (b) 5 x 10⁻⁶ M hemin-treated K-562 cells; (c) uninduced cells. Slots d, e, f were as in c, a, b, respectively, except that cells had been treated with 500 IU/ml HuINF-β.
shown in the center column (cf. Table 2) were thoroughly washed free of IFN and hemin, reseeded in fresh medium, and tested after 24 hr for B+. As further evidence of lack of substantial toxicity of these IFN treatments, data on the right portion of Table 2 show that α-IFN-induced inhibition of Hb production is promptly reversible upon IFN removal.

Essentially identical data, obtained with partially purified β-IFN, are not shown for the sake of brevity. The effects of addition of high doses of γ-IFN have not been studied in view of the unavailability of high titer preparations of this IFN type.

Data shown in Table 2 have been consistently reproduced when a given α-IFN batch was used, but in all fairness do not appear to hold true with every batch received from Dr. Cantell. Human leukocyte IFN consists of an unpredictable mixture of several different molecular species of HuIFN-α, as shown by cloning studies.23,24 It is conceivable that the inhibition of K-562 “induced” erythroid differentiation by high doses of α-IFN is due to one or more such species, the concentration of which may be crucial in a given batch and may vary unpredictably among batches.

**DISCUSSION**

The data presented in this article provide additional evidence pointing to a clear-cut effect of interferons on erythroid differentiation of mammalian cultured cells. In keeping with previous findings in the FLC murine system,13 the administration of low doses of each of the three types of human IFN results in a definite, reproducible, though not massive, induction of differentiation of human K-562S cells (Figs. 3 and 4). When IFN are instead administered to K-562S cultures exposed to either butyric acid or hemin, a pronounced “enhancement” of erythroid differentiation induced by either agent is observed (Figs. 5 and 6). In both instances, Cellogel analysis of Hbs produced in cultures show that IFN treatment results in “enhancing” the production of those Hb types that are already synthesized either constitutively in untreated cultures or upon treatment with the inducers. This is to say that IFN treatment does not result in any specific switch in Hb production.

The specificity of these effects is demonstrated by (A) their reproducibility when pure HuIFN-β is used (Table 1 and Fig. 7), and (B) their abrogation when anti-HuIFN-α serum is also added to untreated cultures given butyric acid and HuIFN-α (Fig. 3).

In striking analogy with findings in the FLC system, the administration of much higher IFN doses to “induced” K-562S erythroid differentiation.

<table>
<thead>
<tr>
<th>α-IFN (IU/ml)</th>
<th>Percent B+ Cells After 4-Day Treatments With 1.2 mM Butyric Acid</th>
<th>5 × 10⁻⁶ M Hemin</th>
<th>Percent B+ Cells 24 hr After Removal of IFN and Hemin</th>
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</thead>
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<tr>
<td>0</td>
<td>63 (58–68)*</td>
<td>71 (63–79)</td>
<td>50 (47–53)</td>
</tr>
<tr>
<td>10,000</td>
<td>42 (36–48)</td>
<td>62 (56–68)</td>
<td>67 (60–74)</td>
</tr>
<tr>
<td>20,000</td>
<td>ND</td>
<td>52 (48–56)</td>
<td>67 (60–74)</td>
</tr>
<tr>
<td>40,000</td>
<td>34 (30–38)</td>
<td>53 (47–59)</td>
<td>69 (62–76)</td>
</tr>
<tr>
<td>80,000</td>
<td>27 (24–30)</td>
<td>44 (39–49)</td>
<td>70 (62–78)</td>
</tr>
<tr>
<td>160,000</td>
<td>15 (13–17)</td>
<td>35 (31–39)</td>
<td>69 (62–76)</td>
</tr>
</tbody>
</table>

*Means (ranges) of 3 experiments.
duced K-562S cultures results in the opposite effect, i.e., inhibition of Hb production, as shown by consistent decrease of B+ cells in the whole cell population (Table 2). No cell mortality is observed in these cultures; furthermore, removal of both IFN and inducer results in the prompt reversibility of the inhibitory effect, indicating that it is not merely due to a toxic effect.

The significance of these findings is strengthened by the pronounced heterogeneity of K-562S cells with respect to their sensitivity to IFN treatment as judged by the establishment of the antiviral state (Fig. 1). The combined evaluation of VSV yields in the supernates of K-562S cells and of the "infectious centers" plating efficiency of IFN-treated K-562S cells clearly shows that roughly 50% of the cells are sensitive to the antiviral action of IFN. It is noteworthy that the same analysis carried out on the murine FLC shows, instead, that the quasitotality of cells develop the antiviral state when given the murine fibroblast IFN. It follows, therefore, that the magnitude of IFN effects on K-562S cells may be larger than it appears from the straight data, as these may reflect the responses of about one-half the population. It is possible, however, that K-562 cell sensitivity to IFN antiviral action does not strictly correlate with their sensitivity to "differentiation-related" IFN effects.

Heterogeneity of this cell line has also been shown with respect to cellular commitment to differentiation, both in regard to the phenotype of Hb synthesis and to the inducibility by butyrate or dimethylformamide. Further, the cells of the subline used in the present study (K-562S) do not clone adequately in semisolid medium. On the other hand, cell cloning by limiting dilutions does not really ensure the stringency required under the circumstances.

Another interesting observation is that of differential Hb "induction" by both butyric acid and hemin, depending on the inducer dose employed (Figs. 5–7). Overloading the gels shows that the lack of three Hb types in untreated or "induced" cultures (Fig. 5) is not a mere quantitative phenomenon. It appears, instead, that Hb synthesis can also be modulated qualitatively in these cultures by simply treating them with low versus optimal inducer dosages.

In conclusion, these data, coupled with those observed in the murine FLC system, emphasize a somewhat "specific" relationship of IFN with erythroid differentiation. In both systems the effects are biphasic according to the IFN dosages employed, giving a hint of a possible regulatory action in vivo. In this respect, it may be more than coincidental that, as suggested by Tim Hunt,28 "reticulocyte lysates so strongly resemble interferon-treated cell extracts in their sensitivity and response to double-stranded RNA that one wonders if the erythocyte pathway of differentiation depends on exposure to a hormone like interferon at some stage." In fact, 2′–5′ A oligonucleotides are formed on incubation of rabbit reticulocyte lysates with ATP and double-stranded RNA. These products are identical to those synthesized by extracts from IFN-treated mouse L cells.27 Likewise, the effects of the oligonucleotide 2′5′ppp(A), on protein synthesis in the highly active rabbit reticulocyte lysate cell-free system and on preincubated and dylized L-cell-free system are closely comparable.

ACKNOWLEDGMENT

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