Isolation of Erythropoietin-Sensitive Cells From Friend Virus-Infected Marrow Cultures: Characteristics of the Erythropoietin Response

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Murine erythroid precursor cells, stimulated to proliferate in vitro in the absence of added erythropoietin (EP) by the anemia strain of Friend virus (FVA), will subsequently respond to EP by complete erythrocyte differentiation. If not exposed to EP, the erythroid cells divide for about 120 hr in culture, and they maintain the potential for full differentiation in response to EP added at any time during the period from 72 to 120 hr. Between 96 and 120 hr of culture without added EP, the EP-sensitive erythroid precursor cells that have formed discrete erythroid bursts can be isolated in relatively large numbers from such cultures by plucking with a Pasteur pipette. The addition of EP initiates the final stages of erythroid differentiation, including heme synthesis in 70%-80% of these isolated cells. With respect to homogeneity of the precursor cells, quantity of EP-responsive cells obtainable, and uniformity of EP responsiveness, this system is uniquely favorable for biochemical studies of the late differentiation effects of EP. The overall changes in gene expression accompanying EP-induced terminal differentiation were examined by two-dimensional gel electrophoresis of proteins labeled for a short time with radioactive amino acids. Several new proteins are synthesized in these erythroid cells during terminal differentiation, but the number is a very small percentage of the total number of proteins being made. Thus, in this system, the effect of EP is to initiate expression of a small group of genes, including those for globins, spectrin, and other proteins involved in the final stages of erythroid differentiation.

Infection of mouse bone marrow cells in vitro with the anemia strain of the Friend leukemia virus complex (FVA) leads to the production of large erythroid colonies (bursts) upon subsequent culture in methylcellulose medium. The proliferation of erythroid progenitors to form these bursts does not require the addition of erythropoietin (EP) to the cultures, but in the absence of added EP, the differentiation of the erythroid cells within the large colonies does not progress to production of heme, globin, globin mRNA, or spectrin. It was shown previously that addition of EP to the cultures at any time up to 72 hr causes the erythroblasts in the bursts to undergo the full program of differentiation, leading to relatively synchronous production of erythrocytes at about 120 hr. However, at 72 hr, the bursts consist of only a small number of cells, and it is not possible to isolate these untreated EP-sensitive cells from other cell types in the cultures in sufficient quantities to study the early biochemical effects of the hormone. In the present study, we discovered that the addition of EP can be delayed until as late as 120 hr, when the hormone will still produce full differentiation of the erythroid precursors. By 120 hr of culture without EP, each erythroid burst contains 3-5 x 10^3 cells, so that it is possible to pluck the bursts manually and isolate several million highly enriched, EP-responsive cells for subsequent biochemical studies of the action of the hormone. This article describes the kinetics of cell multiplication and of the EP response in these cells and provides a perspective of the protein synthetic changes that occur in late erythroid cells in response to the hormone.

MATERIALS AND METHODS

Growth of Friend-Virus-Induced Erythroid Bursts in Semisolid Culture Medium

Bone marrow cells from 8-10-wk-old, phenylhydrazine-treated CD1 mice were infected with FVA and cultured in methylcellulose medium as described previously. At the times indicated in particular experiments, EP was added to replicate cultures at a final concentration of 0.1 U/ml. All of the experiments have been done with sheep plasma EP (4 U/mg protein; Step 3, Connaught Laboratories, Ontario, Canada) and with human urinary EP (1100 U/mg; National Heart, Lung and Blood Institute, Bethesda, Md.), which was free of detectable endotoxin, burst-promoting activity, or granulocyte-macrophage colony-stimulating activities. Identical results were obtained with both EP preparations. For morphological studies of cells in erythroid bursts, the methylcellulose cultures were clotted, fixed, and stained with 3,3' dimethoxybenzidine and hematoxylin.

Isolation of Erythroblast Colonies and Labeling of Proteins

Isolation of erythroblasts from the cell cultures was achieved by plucking the erythroid bursts under a dissecting microscope using a...
drawn-out Pasteur pipette. The isolated cells were at least 90% erythroblasts as determined by Wright-stained cytocentrifuge preparations. These cells were subsequently used for replating in secondary methylcellulose cultures or for incubation with radioactive amino acids to label proteins as described below. For recovery of total cells from methylcellulose cultures in operations where isolation of pure erythroblasts was unnecessary, the methylcellulose medium was diluted at least fivefold with Dulbecco’s minimum essential medium (MEM), and the cells were recovered by low-speed centrifugation. To measure the rate of heme synthesis by cells in methylcellulose culture, incorporation of $^{59}$Fe into protoporphyrin was determined by adding transferrin-bound $^{59}$FeCl$_3$ for 8 hr and then collecting the cells and measuring cyclohexanone-extractable heme $^{59}$Fe.

Radiolabeling of proteins synthesized in isolated erythroblasts was accomplished by resuspending sedimented cells ($0.5-1.0 \times 10^8$ cells/ml) in methionine-free or leucine-free medium containing 10% dailized fetal bovine serum. After 15 min of equilibrium at 37°C, 50 $\mu$Ci/ml of $^{35}$S-methionine (1000 Ci/mmol) or $^{3}$H-leucine (200 Ci/mmol) was added to the medium and incubation at 37°C was continued for 2.5 hr in a humidified 5% CO$_2$ atmosphere. After labeling, the cells were sedimented from the radioactive growth medium and washed once by resuspending and resedimenting them in Dulbecco’s MEM without serum. For determination of the amount of radioactive incorporation into protein, the cells were lysed in 10% trichloroacetic acid, and the precipitated proteins were collected and washed on filter disks (Millipore, type HA). For two-dimensional gel electrophoresis, the cells were disrupted by resuspension in the “lysis buffer” described by O’Farrel. Gel electrophoresis of proteins was conducted by the method of O’Farrel. Gel electrophoresis of proteins was conducted by the method of O’Farrel, with sample lysate derived from 2.5-5.0 $\times 10^7$ cells. After the second-dimension electrophoresis, the gels were fixed with 50% methanol–10% acetic acid, and then processed for fluorography. Each gel was exposed to Kodak XAR-5 film for varying time periods so that signals from all protein spots were within the linear response range of the film on some exposure. Comparisons of the proteins synthesized by erythroblasts treated with EP and those not treated with EP were made by comparing matched x-ray films visually.

RESULTS

Kinetics of Cell Proliferation in Primary FVA-Induced Erythroid Bursts

Recognizable erythroid bursts are first visible in FVA-infected mouse marrow cultures between 72 and 80 hr after initiation of the cultures. Figure 1 depicts the average number of erythroblasts in these bursts as a function of time after they appear. The broken line is a theoretical curve representing exponential proliferation from a single cell with an 8-hr doubling time. This curve generates a cell number at 72–80 hr that is near that expected based on the actual experimental curve. Clearly, by 96 hr, the erythroblast proliferation in the cultures is no longer exponential. After 96 hr, the cell number increase is approximately linear for the next 48 hr and does not increase more than two-fold overall. FVA-infected cultures that received EP 72 hr after infection had the same number of erythroblast cells in the bursts between 96 hr and 168 hr of incubation as the cultures that did not receive the hormone. Addition of EP at times later than 72 hr also caused no increase in the number of cells per burst beyond that observed in bursts developing after virus infection alone (data not shown). Without EP, the erythroblasts lacked hemoglobin, whereas those treated with EP at 72 hr accumulated high levels of hemoglobin by 120–144 hr as indicated by in situ observation and by staining with 3,3’-dimethoxybenzidine.

Duration of EP Sensitivity in FVA-Infected Erythroblasts

$^{59}$Fe incorporation into protoporphyrin to yield heme is a useful indicator of the final phases of erythroid differentiation. It has been previously shown that addition of 0.1 U/ml of EP to the FVA-infected cultures at any time from 0 to 72 hr postinfection (during the period of rapid cell division) causes a well defined peak of iron incorporation at 120 hr due to the relatively synchronous maturation of cells in the FVA-induced erythroid bursts. In the present study, we investigated how long the cells in the bursts retain sensitivity to EP. As can be seen in Fig. 2, if EP is added at 96 or 120 hr instead of 72 hr, peak $^{59}$Fe incorporation is delayed and occurs approximately 48 hr after EP addition. Nevertheless, the peak rates of incorporation per 16-mm culture well are approxi-
The bursts that were already visible in the cultures does not increase much after 120 hr (Fig. 1), the total number of erythroblasts in these cultures. Since the average incorporation per erythroblast in the cell population is similar in all the experiments depicted in Fig. 2. The capacity of cells in the FVA-induced bursts to respond to EP does not extend much beyond 120 hr (data not shown), and by 144 hr of culture without EP, the cells show morphological signs of disintegration.

To show conclusively that the incorporation observed after EP addition at later times (96 or 120 hr) occurred in bursts that were already visible in the cultures, we plucked whole bursts that had been grown 96 or 120 hr without EP, and we recultured them, without dispersion of the cells and without centrifugation, in fresh methylcellulose medium. The cell numbers before and after secondary culture of undispersed bursts (cultures for which heme synthesis is depicted in Table 1) are shown in Table 2. Without EP, the cells of recultured bursts fragmented and died after 48–72 hr of subculture. Table 1 shows the results of incorporation measurements on the secondary cultures of undisrupted plucked bursts that were incubated with or without EP. The large bursts plucked at 96 or 120 hr and recultured with EP responded with a large increase in incorporation into protoporphyrin, corroborating the morphological and benzidine staining results described above. Similar numbers of cells isolated after 96 hr or 120 hr in primary culture incorporated similar amounts of iron. This indicates, as the data of Figs. 1 and 2 suggest, that the ability of the erythroblasts to synthesize heme in response to EP does not change appreciably if EP is added between 96 and 120 hr of culture. Bursts recultured in the absence of EP did not have a similar increase in the incorporation of Fe.

The above results demonstrate that the erythroblasts in FVA-induced erythroid bursts can continue to proliferate to a limited extent after 96 hr, and they remain capable of complete differentiation if they are exposed to EP up to 120 hr after initiation of the original culture. In addition, the cells can be isolated from the primary cultures at 96–120 hr, and they are able to withstand the isolation procedure and still respond to EP after placement in secondary culture.

### Proliferation Potential of Isolated FVA-Induced Erythroid Cells

We investigated the proliferation of erythroid burst cells isolated at 120 hr when recultured in fresh medium. The cell numbers before and after secondary culture of undisrupted bursts (cultures for which heme synthesis is depicted in Table 1) are shown in Table 2. As was the case in primary cultures after 120 hr, the undisrupted erythroblasts did not increase more than twofold upon reculturing in fresh methylcellulose

#### Table 1. Incorporation Into Protoporphyrin in Undisrupted Erythroid Bursts Plucked at 96 or 120 hr After Culture

<table>
<thead>
<tr>
<th>Time of Harvest From Primary Culture</th>
<th>EP Added to Reculture Medium</th>
<th>59Fe (cpm) Incorporated/16-mm Well During the Indicated Periods After Reculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 hr</td>
<td>None</td>
<td>12–24 hr</td>
</tr>
<tr>
<td>96 hr</td>
<td>0.1 U/ml</td>
<td>ND</td>
</tr>
<tr>
<td>120 hr</td>
<td>None</td>
<td>ND</td>
</tr>
<tr>
<td>120 hr</td>
<td>0.1 U/ml</td>
<td>166 ± 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>530 ± 35</td>
</tr>
</tbody>
</table>

An aliquot of the plucked bursts was disrupted to single cells for counting with a hemocytometer. Each 16-mm culture well then received an aliquot of undisrupted plucked bursts containing 0.5 ± 0.1 × 10⁶ cells at initiation of the reculture period. All data are mean cpm per culture well ± 1 SD obtained from triplicate cultures.
medium. Because the limited increase in cell numbers in the above experiments could be the result of medium depletion in the primary culture (Fig. 1), or perhaps diffusion limitations in undispersed bursts in secondary culture, we also recultured plucked erythroblasts after complete dispersal to single cells and determined the proportion of such cells that formed cell colonies during 48 hr further incubation (Table 2). In these experiments, about one-half of the replated cells, which received EP, did not divide, while one-half of the cells formed colonies of two or more cells within 48 hr. After increasing about threefold during 48 hr of incubation with EP, the total cell numbers did not increase further, and the cells in the colonies deteriorated thereafter. Without EP, no increase in the cell number was evident, and only 25%-35% of the cells divided before beginning to deteriorate after 48 hr of subculture. The greater effect of EP on increase in cell number in secondary cultures of dispersed cells as opposed to undispersed cells could be secondary to a greater availability of EP to the dispersed cells that enhanced their capacity to divide or reduce premature death. Nevertheless, these experiments show that the dispersed FVA-induced erythroid cells have a visible but limited division potential in this type of culture environment whether or not they mature into erythrocytes in response to EP.

Proportion of the Isolated Erythroblasts That Respond to EP

The proliferation studies suggest that many cells in the FVA-induced bursts may not divide after about 96 hr in culture, and yet, almost all cells in the bursts appear to accumulate heme (as indicated by benzidine staining) in response to EP added as late as 120 hr. Because some cells in the erythroid bursts might fail to divide, die, and disappear only to be replaced by an approximately equal number of newly divided, differentiating cells in a steady-state situation, we sought to determine the proportion of erythroid cells plucked at 120 hr from primary cultures that respond directly to EP with increased heme synthesis. Secondary cultures of erythroblasts that had been dispersed and replated as single cells and then incubated in the presence of the hormone for 24 or 48 hr were clotted and stained with benzidine. Almost all of the cells that divided and produced colonies, including 2-cell colonies, became heme positive. Also, at least 20%-30% of the cells that remained single in the secondary cultures accumulated heme as indicated by staining with 3,3'dimethoxybenzidine. Thus, at least 70%-80% of the cells that were isolated from erythroid bursts of primary cultures at 120 hr responded to EP by differentiating into heme-synthesizing late erythroblasts. This is a minimum estimate of the erythroid cells that synthesize heme because of two factors. First, a small portion (about 10%) of the cells that we isolate by plucking bursts are not erythroblasts; most of these contaminating cells are macrophages, as indicated by Wright-stained cytocentrifuge preparations. Second, about 10% of the plucked dispersed cells are not viable at the time of replating, as indicated by Trypan blue exclusion studies, and yet they may be scored as single heme-negative cells in experiments estimating the percentages of cells forming colonies and synthesizing heme.

Protein Synthesis Changes During the Terminal Phase of Erythroid Differentiation

To get a perspective on the process of differentiation, it is useful to gain a description of the number of
genes whose change in expression constitutes the process. The in vitro FVA-induced erythropoiesis system permits biochemical comparisons between erythroblasts in the process of terminal differentiation in response to EP and erythroblasts that fail to undergo certain aspects of the differentiation program in the absence of the hormone. In previous work, we showed that EP induces de novo synthesis of globin and spectrin, two erythrocyte-specific proteins, during terminal differentiation of erythroblasts in this in vitro system. We have now studied protein synthetic changes during EP-induced differentiation in this system in more detail. First, we determined whether addition of EP to developing bursts at 72 hr of culture and the ensuing erythroid differentiation that occurs during the subsequent 48 hr has an effect on the overall rate of protein synthesis in the cells as compared to control erythroblasts of bursts not receiving EP. FVA-infected cultures were established, and 0.1 U/ml of EP was added to one-half of the wells at 72 hr. At 120 hr of culture, erythroblasts were plucked from both culture groups. One million erythroblasts isolated from cultures treated or untreated with EP were washed and labeled with 1H-leucine, as described in Materials and Methods. After incubation with 1H-leucine, the cells were washed by centrifugation and the incorporation of radioactive leucine into protein was determined by precipitation of the proteins with trichloroacetic acid followed by scintillation counting. The results of several experiments showed that there was no difference at 120 hr between the incorporation of leucine by erythroblasts that had received EP at 72 hr and those that had not. The typical levels of 1H-leucine incorporation by these cells was 1.5 x 10^6 cpm/1.0 x 10^6 cells.

We next determined the percentage of the 1H-leucine that was incorporated into hemoglobin in the erythroblasts that received EP. This was accomplished by resolution of the globin proteins from the bulk of other cell proteins by electrophoresis on Triton-acid-urea gels, followed by solubilization of the appropriate gel portions in tissue solubilizer (NCS, Amersham Corporation) and scintillation counting. In erythroblasts that received EP at 72 hr and were isolated and labeled (2.5 hr) after 120 hr in culture, 30% of the total incorporated 1H-leucine was present in the 3 major globin polypeptides, α, βmin, and βmaj. Thus, even though these cells are at a late stage of erythrocyte differentiation, as evidenced by enucleation of 30%–50% of the cells, synthesis of proteins other than globins still accounts for the major portion of their protein synthesis. Erythroblasts from FVA-induced bursts that are not exposed to EP do not synthesize appreciable globin polypeptides.

Two-dimensional gel electrophoresis of pulse-labeled total cell proteins was used to examine the extent of changes in the synthesis of nonglobin proteins caused by EP in the cells of the erythroid bursts. The gels of Fig. 3A and 3B, respectively, are representative gels that reflect the protein synthesis of erythroblasts in FVA-induced plucked bursts (plucked at 120 hr) that did or did not receive 0.1 U/ml of EP at 72 hr of culture. After isolation, these cells were labeled for 2.5 hr with 35S-methionine. Bearing in mind that these burst cells, with or without EP, are dramatically different in globin synthesis, heme synthesis, and spectrin synthesis and in morphological appearance, it is very striking that the patterns of proteins resolved by the gels appear very similar. Although about 350 proteins are clearly resolved and have been analyzed in such gels, only 5 or 6 new proteins (designated by arrows in Fig. 3A) are consistently observed in cells undergoing terminal differentiation in response to EP in comparison to those cells not treated with EP. The designated protein of molecular weight 260,000 migrates at the position of a spot immunoprecipitated by antispectrin antiserum (data not shown). The designated protein of molecular weight 16,000 comigrates with a globin polypeptide (presumably βmaj) from mature erythrocytes. The other new proteins synthesized in response to EP have not been identified with known proteins, but they are not due to low-level contamination of our cell population by none erythroid cells because they do not appear in two-dimensional gels of granulocyte or macrophage colonies isolated from our marrow cultures. No visible proteins were missing in EP-treated cells that were present in untreated cells. The above assessment of protein synthesis differences is based on a visual inspection of five pairs of two-dimensional gels representing five separate culture experiments in which half the wells received EP and the other half did not. There was some variation in these experiments in terms of clarity of appearance of quantitatively minor proteins. This variation reflected biologic variations, since multiple electrophoretic separations conducted on a given sample always gave identical patterns. Thus, in a given pair of gels, several more proteins than indicated above occasionally appeared to be different between hormone-treated and untreated cells. Checking of the proteins in question on multiple gels of independently prepared samples was thus necessary in a few instances to determine that these proteins did not represent the initiation of new protein synthesis by the hormone. We have also made similar comparisons of proteins using 3H-leucine as the radioactive precursor and, in addition, have run second-dimension gels of various acrylamide concentrations to resolve better high and low molecular weight ranges. The above conclusions have been verified under all conditions used.

The similarity of the pattern for erythroblasts with
Fig. 3. Effect of EP on protein synthesis in erythroblasts of isolated, FVA-induced bursts. Two-dimensional gel electrophoresis was conducted by the method of O'Farrell using 5%–15% polyacrylamide-sodium dodecyl sulfate gradient gels for the second dimension. 14C-labeled molecular weight marker proteins (New England Nuclear, Boston, Mass.) were run in a parallel gradient gel and processed for fluorography in a manner identical to the experimental gels. Marker proteins consisted of carbonic anhydrase (mol wt 30,000), bovine serum albumin (mol wt 66,200), phosphorylase B (mol wt 92,500), and myosin (mol wt 200,000). (A) Solubilized erythroblasts from cultures that received 0.1 U/ml of EP at 72 hr postinfection. The erythroid bursts were plucked from primary culture at 120 hr and the cells were labeled with 35S-methionine for 2.5 hr as described in Methods. (B) Solubilized erythroblasts that were processed as above except that they received no EP at 72 hr. Arrows in part A designate several proteins that were observed only in cells that received EP.
and without EP suggests that the group of genes turned on by EP late during differentiation represents a very small fraction (on the order of 2%) of the proteins made in erythroblasts. Also, there is no rapid, differential cessation of synthesis of any particular proteins, except that globin synthesis constitutes an increasingly larger portion of the protein synthetic process in maturing erythroblasts.

The proteins resolved by these gels do not represent all of the proteins of the cells. Some proteins have isoelectric points outside the pH range of the isoelectric focusing gels, other proteins are insoluble in the buffer system, and still others have little or no methionine or leucine or are present in too small a quantity to detect. Nevertheless, there is no reason to suspect that the proteins that do appear on the gels are not a statistically representative sample of the total cell proteins.

**DISCUSSION**

Previous reports have shown that in vitro infection of mouse bone marrow cells with FVA and subsequent culture of the cells in semisolid medium leads to proliferation of erythroid precursors into large colonies. The cells of the FVA-induced colonies, unlike those induced by the polycythemia strain of Friend virus (FVP), do not undergo full differentiation into erythrocytes unless EP is added to the in vitro cultures. Our present work demonstrates that this in vitro, FVA-induced, erythropoiesis system can be used to isolate a highly enriched population of EP-sensitive progenitor cells suitable for various types of biochemical studies, including examination of the rapid effects of EP on target cells. The FVA-infected erythroid progenitors proliferate for up to 144 hr without EP, yielding colonies of $2 \times 10^3$ to $5 \times 10^3$ cells that can be plucked manually using a dissecting microscope and a modified Pasteur pipette. The key to the usefulness of this system is the fact that the erythroid cells maintain the capability for full differentiation in response to EP for about 120 hr after initiation of the primary culture. This allows cultures to generate bursts of sufficient size so that plucking is possible and larger numbers of cells can be collected. Erythroblasts plucked after 96–120 hr of culture constitute a population of developmentally synchronous cells (about 90% erythroblasts), which in homogeneity of cells, quantity of EP-responsive cells, and availability of control cells that have not received hormone treatment is uniquely favorable for study of EP action. This system may provide additional information about erythroid differentiation to that obtained from studies of murine erythroleukemia cell lines that do not exhibit EP sensitivity.

By manual plucking of FVA-induced erythroid bursts at 120 hr of culture, we can obtain 10–20 x 10^6 erythroblasts of 90% purity from infected marrow cells of 20 mice. For experiments that do not require such a high degree of purity of the erythroblast population, one can simply recover all of the nonadherent cells in the cultures by dilution of the methylcellulose medium and sedimentation of the cells. The cell population acquired in this manner is approximately 50% erythroblasts, and about 2 x 10^6 cells can be recovered rapidly from cultures originated from 20 mice.

An important concern about the isolated erythroblasts was the uniformity of their early biochemical responses to EP. Although secondary culture of the whole bursts in the presence of EP appeared to lead to heme synthesis by almost all cells after 24–48 hr of subculture, it was conceivable that there was rapid turnover of cells within the population and that terminal differentiation was restricted to only those cells that proliferated. We thus did experiments to examine the proliferative capacity of the isolated erythroblasts and to determine what portion of the cells we isolate by plucking colonies from primary culture actually differentiate in response to EP. The most revealing of these experiments were those in which isolated single erythroblasts were analyzed for their ability to divide (Table 2) and simultaneously for their ability to synthesize heme in response to EP. About 50% of the isolated cells divided at least once, and those that did so gave rise to fully differentiated colonies of erythroid cells. Of the cells that did not divide, 20%–30% synthesized heme as assessed by benzidine staining. Thus, we know that at least 70% of the isolated cells can respond directly to EP. This percentage may be an underestimate, due to some damage to isolated cells by manipulations necessary to disperse bursts into single cells. While many cells isolated after 120 hr of primary culture are capable of dividing at least once, they do not seem to possess the potential in vitro for more than 2 or 3 more cell divisions. This conclusion is borne out by all of our measurements of cell number increases in primary or secondary cultures. The FVA-infected erythroid precursors apparently proliferate exponentially for at least 72 hr, but thereafter, proliferation slows dramatically. This does not seem to be due to medium depletion, because secondary culture of the cells does not lead to a return to exponential proliferation. It is very likely due to differentiation-related changes in the cells causing them to lose the ability to divide further. In an earlier study of EP-induced differentiation of FVA-infected erythroid progenitors, we described several dramatic changes that occur in these cells in response to EP: synthesis of large amounts of hemoglobin, synthesis of spectrin, and extrusion of the nuclei.
from the cells. In the present study, we examined the overall protein synthetic changes that occur in the cells during EP-induced terminal differentiation. The results of our two-dimensional gel studies represented in Fig. 3 (A and B) demonstrate that most of the proteins made in late FVA-induced erythroblasts are made before the addition of EP in our system and that only a small group of late genes, including those for hemoglobin, spectrin, and a few other unidentified proteins, account for the terminal maturation events observed when EP is added to the cultures. A similar finding has been reported using two-dimensional gels to study Friend erythroleukemia cell lines before and after induction with DMSO. These observations are intriguing in that they raise two possibilities: (1) if erythroid differentiation from stem cells involves turnon of a large number of erythroid-specific genes and turn-off of other genes, then most of these changes in expression must occur almost exclusively in earlier developmental stages, or (2) differentiation of erythrocytes, granulocytes, megakaryocytes, etc., from a common stem cell may involve relatively few gene expression changes superimposed on a much larger background of genes expressed commonly in a large number of cell types. We are now examining these possibilities further by conducting two-dimensional gel analyses of proteins synthesized in granulocyte precursors, macrophage precursors, and in fibroblast-like cell lines. These comparisons will be published in detail elsewhere. It is clear that at least 50% of the major proteins synthesized in the erythroblasts are synthesized at similar rates in all of the other cell types mentioned above and that the “granulocytic” cells contain an even higher proportion (60%–70%) of labeled proteins that appear identical to those in erythroblasts. We have not yet analyzed the relatively minor proteins. However, even these preliminary results show that erythroblasts that are very late in the differentiation pathway continue to make most of the major proteins that are made in a variety of other cell types.

REFERENCES

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