Selective Inhibition of the Growth of Human Erythroid Bursts by Monoclonal Antibodies Against Transferrin or the Transferrin Receptor

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The relative requirements of colonies derived from erythroid (BFU-E) and myeloid (CFU-c) progenitors for transferrin were examined using monoclonal antibodies directed against the transferrin molecule (TF-6) or its cell surface receptor (TFR-A12, TFR-1-2B). Growth of erythroid bursts was profoundly reduced at concentrations of all three antibodies that had no effect on CFU-c-derived colonies. When TFR-1-2B was layered over cultures established one to seven days previously, further burst development was inhibited, and degeneration of early erythroid colonies was observed. Addition of erythropoietin augmented transferrin receptor expression on cells harvested after 1 to 2 weeks in culture and analyzed by flow cytometry. Recombinant human erythropoietin gave results comparable to those obtained in experiments using human urinary erythropoietin. Analysis of erythroblasts plucked directly from culture plates confirmed the presence of transferrin receptors on BFU-E-derived colonies. Thymidine incorporation was maximal early in the second week of culture and coincided with high transferrin receptor expression. These data demonstrate that transferrin must be available into the second week of culture to support the growth and differentiation of BFU-E-derived erythroid bursts, that the generation of erythroid colonies from BFU-E is more dependent on transferrin than myeloid colony formation from CFU-c, and that erythropoietin modulates the expression of transferrin receptors on growing bursts.

MATERIALS AND METHODS

Production and characterization of MoAb. MoAb TF-6, which is specific for human transferrin, was produced by standard fusion techniques. Murine plasmacytoma cell line NS1 was fused to spleenocytes from mice immunized and boosted with iron-saturated human transferrin. Hybridomas were screened by transferrin-specific enzyme-linked immunosorbent assay (ELISA). Cloned cell line TF-6 produced an IgG monoclonal antibody that immunoprecipitates human transferrin. The antibody also recognized transferrin bound to cell surface.

MoAb TFR-1-2B13 and TFR-A12 (originally designated 454-A12),14, which recognize the transferrin receptor, have been described previously. Both antibodies bind to cell surface transferrin receptors, immunoprecipitate the 94,000-molecular weight (mol wt) transferrin receptor, and recognize different sites on the receptor distant from the actual transferrin binding site. TFR-1-2B is of the IgG2a isotype and TFR-A12 of the IgG1 isotype. Ascites containing TF-6, TFR-A12, and TFR-1-2B was purified by Seph-Protein A affinity chromatography and by diethylaminoethyl (DEAE) ion-exchange chromatography.

MoAb MY715 was obtained from Coulter Immunology (Hialeah, Fla). A MoAb that recognizesDR antigens was obtained from Becton Dickenson (Mountain View, Calif).

Culture of myeloid and erythroid progenitors. Approximately 10 mL of whole blood was collected from the cut end of the...
umbilical cords of healthy neonates into 10 mL of heparinized culture medium consisting of 20% fetal calf serum and 1% bovine serum albumen in RPMI 1640 medium (GIBCO, Grand Island, NY). Samples were kept at room temperature for up to 16 hours prior to isolation of mononuclear cells by Ficoll-Hypaque density centrifugation. Mononuclear cells were aspirated from the interface, and the remaining erythrocytes were lysed in ammonium chloride solution. After washing, the cell population was resuspended in Iscove's modified Dulbecco's medium (IMDM) for plating in culture medium consisting of 0.8% methylcellulose and supplemented with 35% fetal calf serum (Flow Laboratories, McLean Va.; lot 29107036), 1.2% bovine serum albumen, 1-glutamine, and final concentrations of 10^{-3} Mol/L 2-mercaptoethanol and 0.01% iron-free human transferrin (Sigma Chemical Co, St Louis). Commercial colony-stimulating factor (GCT medium, GIBCO; lot 18K6242) was added to all cultures. Human urinary erythropoietin was obtained from the British Columbia Cancer Centre, Vancouver, BC. In some experiments, recombinant human erythropoietin with a specific activity of > 70,400 U/mg of protein (Amen Corp, Thousand Oaks, Calif) was used instead of EPO purified from human urine. Duplicate 30 x 15-mm plates (Falcon 3001, Oxnard, Calif) containing 1 mL of methylcellulose culture medium and a final concentration of 5 x 10^5 cells per plate were cultured. After 14 days at 37 °C in a 5% CO2 incubator, progenitor colonies were enumerated by indirect light microscopy according to the criteria of Gregory and Eaves for erythroid bursts and Pike and Robinson for CFU-c-derived colonies.18

A number of precautions were taken to ensure consistency of the culture conditions. Single lots of fetal calf serum and GCT medium were used in all experiments. Stock methylcellulose culture medium was prepared in 100-mL lots, tested for its ability to support the growth of BFU-E and CFU-c, and stored at -70 °C in test tubes containing 2.5 mL each before use. For individual experiments, all containing BFU-E and CFU-c, and stored at -70 °C in test tubes were used in all experiments. Stock methylcellulose culture medium (1MDM) for plating was prepared in parallel from the same lot. IMDM was carefully layered over each 1-mL plate.

To assess the effects of early antibody exposure on colony development, a series of experiments was carried out in which primary cultures established as previously described were incubated at 37 °C for up to five days. These cells were then harvested and replated in secondary cultures as follows: primary dishes were washed vigorously with 15 mL of IMDM and centrifuged at 2,000 rpm for 15 minutes. The pellets were washed three times each in a large excess of IMDM to dilute out residual antibody and methylcellulose. Following the final wash, the cell pellet was resuspended in 0.3 mL of IMDM and added to 2.5 mL methylcellulose tubes from the same lot, mixed, and replated. Secondary plates were scored for the presence of BFU-E- and CFU-c-derived colonies 14 days after the original cultures had been established.

All studies using human umbilical cord blood were approved by the Committee for the Protection of Human Subjects at the Naval Hospital, Oakland, Calif, and by the Naval Health Sciences Education and Training Command. Preparation of mononuclear cells for immunofluorescent analysis. Cultured cells were removed from methylcellulose after seven to 14 days by serially washing 35-mm plates with a total of 12 mL of medium. Cells were centrifuged at 1,500 rpm for ten minutes, and the pellet was resuspended in IMDM and washed twice to remove any residual methylcellulose. Mononuclear cells obtained in this manner were used in studies of surface antibody binding and thymidine incorporation as will be described. Purified populations of erythroblasts were obtained by plucking individual bursts under indirect microscopy using a 10-μL micropipette. Wright-Giemsa staining confirmed that more than 95% of cells obtained in this manner were erythroblasts.

Cell sorter analysis. An EPICS V sorter (Coulter Electronics, Hialeah, Fla) equipped with a 78-μm nozzle and a 5-W argon laser was used for cell surface marker analysis. The 488 nmol/L line at 1-W light-regulated power was used for fluorescein excitation. Cells were stained with monoclonal antibodies by the method of Loken and Stall.17 Fluorescein isothiocyanate conjugate goat F(ab')2 anti-γ and anti-μ reagents were purchased from Tago (Burlingame, Calif). To eliminate dead cells from analysis, 10 μL of propidium iodide (Sigma Chemical Co, 5 μg/mL in distilled water) was added to 1 mL of cells in phosphate-buffered saline (PBS) with 0.1% gelatin and 0.2% NaN3, and fluorescence was measured on propidium iodide (PI)-negative cells. The percentage of cells labeled by individual antibodies was determined by a computer program that uses a least-squares algorithm for immunofluorescent histogram analysis (Coulter Electronics, Hialeah, Fla).

Proliferation assay. Mononuclear cells were removed from methylcellulose and washed as previously described. Cells were immediately transferred to flat-bottomed microtiter plates (1 x 10^5 cells per well) and incubated for six hours at 37 °C in the presence of tritiated thymidine (0.5 μCi/well) before harvesting. Wells were aspirated using a PHD cell harvester (Cambridge Technology), and Scintiverse scintillation fluid (Fisher Scientific, Fairlawn, NJ) was added to vials, which were then counted on a Minibeta 1212 (LKB, Gaithersburg, Md) liquid scintillation counter. A total of six wells was cultured in all proliferation studies; the results of individual experiments were obtained by eliminating the highest and lowest counts and calculating a mean for the four remaining wells.

Statistical analysis. The effect of various concentrations of TF-6, TFR1-2B, and TFR-A12 on colony growth was assessed by comparing the growth in plates containing antibody with plates prepared in parallel from the same cord blood specimen to which no antibody was added. Growth in plates without antibody was defined as representing 100% of control numbers for that experiment and was compared with the growth in plates to which antibody had been added. The mean percent inhibition and SD were calculated by pooling the data derived from individual experiments. All statistical comparisons were made using Student's paired t test.

RESULTS

Growth of progenitors in the absence of antibody. Cord blood mononuclear cells cultured in the presence of 2U EPO/mL without antibody yielded a mean of 146 CFU-c (range 51 to 254) and 122 BFU-E (range, 23 to 220) derived colonies per 10^5 cells plated. The plating efficiency for erythroid progenitors grown from cord blood mononuclear cells was similar to the results of others10 and to what we have reported previously.18 In the absence of added EPO, no BFU-E colonies were observed while myeloid colony growth was unchanged.

Selective inhibition of BFU-E growth by MoAb. Addition of TFR1-2B at the time cultures were established profoundly reduced the growth of BFU-E-derived colonies as shown in panel A of Fig 1. Erythroid colony numbers were reduced to less than 20% of the control by 0.1 μg/mL of TFR1-2B; no bursts were observed at concentrations >0.5 μg/mL. In contrast, a significant decrease in the number of
CFU-c-derived colonies did not occur until a TFR1-2B concentration of 10.0 µg/mL was reached. Even at 50.0 µg/mL of antibody, growth of CFU-colonies remained greater than 50% of control values.

A somewhat different pattern of inhibition was observed in cultures to which TFR-A12 was added (Fig 1, panel B). As was the case with TFR1-2B, erythroid bursts were far more sensitive to the inhibitory effects of antitransferrin receptor antibody than were CFU-c-derived colonies. Reduction in CFU-c colony numbers occurred at TFR-A12 concentrations of 10.0 and 50.0 µg/mL (.01 < P < .05), but overall growth was greater than 50% of control values, even at the highest dose of antibody tested. In contrast, low doses of TFR-A12 (0.1 to 0.5 µg/mL) decreased BFU-E-derived colonies by 50% to 80%. Most of the erythroid colonies present in cultures carried out in the presence of these low doses of antibody were large and contained abundant hemoglobin. Morphologically, they were similar to the well-developed burst shown in the first panel of Fig 2. At concentrations of TFR-A12 above 1.0 µg/mL, the number of bursts did not decrease further; however, the erythroid colonies present were poorly developed and contained very little visible hemoglobin. A representative burst grown in the presence of 5.0 µg/mL of TFR-A12 is shown in the second and third panels of Fig 2.

Results of experiments using TF-6, the MoAb against the transferrin molecule, are summarized in panel C of Fig 1. The effects on colony growth of antibody against transferrin were similar to those of antibodies against its cell surface receptor. Development of bursts was inhibited at significantly lower concentrations of TF-6 than that of CFU-c. Growth of erythroid progenitors was normal at 0.1 and 1.0 mg/mL of TF-6 but was completely abrogated by 5.0 µg/mL. A significant decrease in the number of CFU-c was not observed until an antibody concentration of 10.0 µg/mL was reached, and growth remained greater than 60% of control plates at 50.0 µg/mL.

**Kinetics of BFU-E inhibition by TFR1-2B.** Addition of antibodies against transferrin or its cell surface receptor at the time cultures were established markedly reduced the proliferation and differentiation of erythroid progenitors, as was shown. We next investigated the effect of deferring the addition of antibody until one to seven days after the cells were initially plated. For these experiments, we selected a concentration of TFR1-2B (3.0 µg/mL) that was known to completely inhibit the development of BFU-E-derived colonies if added on day 0. We observed a >90% reduction in the number of bursts when TFRI-2B was added as late as day 7 (Fig 3). This finding is especially notable because immature bursts containing few cells and small amounts of hemoglobin become visible between day 5 and day 7. Most of these colonies had degenerated by day 14 in plates to which TFRI-2B had been added. The observation that transferrin must be available into the second week of culture for optimal growth and development of BFU-E-derived progenitor colonies led us to conduct experiments in which mononuclear cells were harvested from methylcellulose to analyze transferrin receptor expression and proliferation.

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**Fig 1.** Effect of MoAb on progenitor colony growth. At final concentrations between (A) 0.1 and 50.0 µg/mL, TFR1-2B, and (B) TFR-A12 significantly reduced BFU-E-derived colony growth (P < .001 compared with control cultures for all doses tested). Development of bursts proceeded normally in the presence of 0.5 and 1.0 <µg/mL of TF-6; (C) however, 5.0 µg/mL completely abrogated erythroid colony growth. All three antibodies profoundly decreased the number of bursts at concentrations that had no effect on CFU-c-derived colonies. Some reduction of CFU-c numbers occurred at 10.0 and 50.0 µg/mL (.01 < P < .05 for all three antibodies). Each point represents pooled data from five to 13 experiments. •—CFU-C; O—BFU-E.
Fig 2. Alteration of burst morphology by TFR-A12. (A) normal erythroid burst containing large subcolonies appears dark because of abundant hemoglobin production (50 x). (B) Representative burst from the same cord blood specimen grown in the presence of 5 μg/mL of TFR-A12 (50 x). Individual bursts were poorly developed and contained small subcolonies (C). Higher power view of burst from (B) (original magnification x 2100 x; current magnification). Subcolonies contain few cells; little hemoglobin was visible in individual colonies.

Replating experiments and preincubation with MoAb. In the experiments just described, antibodies were consistently present in all cultures throughout the second week, a period of rapid proliferation and hemoglobinization in developing bursts. To more directly assess the sensitivity of BFU-E to antitransferrin receptor antibodies early in culture, mononuclear cells were harvested after 1, 3, or 5 days, washed, and replated in secondary cultures. For these studies, final concentrations of TFR-A12 and TFR1-2B of 1.0 μg/mL were used in both primary and secondary cultures. As shown in Table 1, inhibition of BFU-E colony development was dependent upon the duration of antibody exposure. Cultures that contained TFR1-2B or TFR-A12 for one day yielded normal numbers of BFU-E at 2 weeks. In contrast, when replating was delayed as long as five days, a marked reduction in BFU-E growth was observed (Table 1). The few colonies that were derived from secondary cultures established on day 5 without antibody from plates initially cultured with antibody were poorly developed and resembled those shown in the second and third panels of Fig 2. Growth of CFU-c was completely unaffected by these relatively low concentrations of antibody and was similar in cultures replated throughout the first five days.

Because the methylcellulose medium used to culture progenitor colonies (35% fetal calf serum) contained large amounts of transferrin, it was possible that the pronounced
difference in the growth of erythroid and myeloid colonies might be due to a rapid uptake of transferrin by CFU-c rather than to a true difference in the intrinsic sensitivity of the target cells to MoAb. To investigate this possibility, cord blood mononuclear cells were incubated overnight in IMDM containing either no antibody or 10 μg/mL of TF-6, TFR1-2B, or TFR-A12. The cells were then washed thoroughly and cultured in methylcellulose. Preincubation of mononuclear cells with antibody resulted in no inhibition of either erythroid or myeloid colony growth at 2 weeks unless additional antibody was added at the time of plating in methylcellulose (data not shown).

Observations using the antibody TF-6, which does not bind to cell membranes, suggested that our results could not be explained by nonspecific, complement-mediated cytotoxicity. To further evaluate this question, methylcellulose medium was heat inactivated for two hours at 56 °C immediately prior to the addition of cells, antibody, and EPO. The inhibitory effects of TFR1-2B, TFR-A12, and TF-6 on the growth of BFU-E-derived colonies were identical in control cultures grown with and without EPO (data not shown). MoAb antibody binding and proliferative response of cultured mononuclear cells. Surface labeling of transferrin receptors and of the myeloid differentiation antigen MY7 was carried out on cells harvested from methylcellulose. Addition of EPO at the time cultures were established markedly increased the expression of transferrin receptors on mononuclear cells analyzed after seven to 14 days (Table 2). In contrast, the higher proportion of MY7-positive cells in cultures grown without EPO reflects a predominance of myeloid progenitor colonies. Histograms from an experiment in which cells from a single cord blood specimen were labeled with TFR1-2B and MY7 after nine and 14 days in culture are presented in Fig 4. Both the percentage of cells with detectable transferrin receptors and the density of labeling with antireceptor antibodies were greater in cultures harvested on day 9. Because the major difference between cultures grown with and without EPO was the presence of BFU-E-derived colonies in the former, we inferred that erythroid progenitors were the population with high transferrin receptor expression. This was confirmed by analysis of erythroblasts plucked from mature bursts after 14 days in culture (data not shown).

Table 1. Growth of Erythroid Bursts Following Replating

<table>
<thead>
<tr>
<th>Antibody Added (±) to</th>
<th>Number of BFU-E Colonies per 10⁶ Cells in Cultures Replated on</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 1 (n = 6)</td>
</tr>
<tr>
<td>−−</td>
<td>133 ± 28</td>
</tr>
<tr>
<td>+−</td>
<td>122 ± 22</td>
</tr>
<tr>
<td>−+</td>
<td>26 ± 8†</td>
</tr>
<tr>
<td>++</td>
<td>16 ± 7†</td>
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</tbody>
</table>

Symbols indicate values significantly different from reference cultures replated on the same day containing no antibody in either primary or replated cultures. Data from these reference cultures are plotted on the top line. * = P < .05; † = P < .01; ‡ = P < 0.005. Values for day 1 and day 5 were analyzed by the paired t test; values for day 3 were from one-way analysis of variance (ANOVA) because of the small number of data points.

Table 2. Surface Binding of Antibody by Cord Blood Mononuclear Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Days in Culture</th>
<th>Percentage Positive With Antibody</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>TFR1-2B</td>
<td>TFR-A12</td>
</tr>
<tr>
<td>1</td>
<td>7 + EPO*</td>
<td>32.2</td>
</tr>
<tr>
<td></td>
<td>−EPO</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>7 + EPO</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>−EPO</td>
<td>3.7</td>
</tr>
<tr>
<td>3</td>
<td>9 + EPO</td>
<td>84.4</td>
</tr>
<tr>
<td></td>
<td>−EPO</td>
<td>14.4</td>
</tr>
<tr>
<td>4</td>
<td>14 + EPO</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>−EPO</td>
<td>0.7</td>
</tr>
<tr>
<td>5</td>
<td>14 + EPO</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>−EPO</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>−EPO</td>
<td>16.0</td>
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</tbody>
</table>

Abbreviation: ND, phenotype not determined.

* + EPO and − EPO refer to cells removed from plates cultured in the presence (+) or absence (−) of erythropoietin.
Fig 5. Proliferation of cells grown with (A) EPO+ and without (B) EPO− erythropoietin. Serial analysis of thymidine incorporation of cells cultured from a single cord blood specimen pulsed after four to 14 days in culture. The high level of thymidine incorporation early in the second week of culture coincides with a rapid increase in the cellularity and hemoglobinization of bursts. Flow cytometry of cells harvested from the same experiment (Fig 4) showed that transferrin receptor expression on days 9 and 14 paralleled changes in thymidine uptake.

Fig 4. Flow cytometry of cells grown with (A) EPO+ and without (B) EPO− erythropoietin. The heavy line represents binding by fluorescein-labeled TFR1-2B or MY7; the light line shows a fluorescein-labeled negative control antibody. Transferrin receptor expression was markedly increased on cells harvested from EPO+ plates; in contrast, a higher percentage of EPO− cells were labeled by MY7. Enumeration of progenitor colonies on day 14 revealed 74 CFU-c− and 110 BFU-E−derived colonies per 10⁸ cells cultured on EPO+ plates and 76 CFU-c and no BFU-E colonies on EPO− plates. Since cells from the same cord blood were analyzed after nine and 14 days in culture, the data also suggest downregulation of transferrin receptors on mature bursts. The percentage of positive cells in each study is given in Table 2 as experiment 3.

To determine whether the increase in transferrin receptor expression in the presence of EPO was accompanied by cellular proliferation, thymidine uptake by cultured cells was examined. As depicted in Fig 5, maximum thymidine incorporation was observed in plates containing EPO+ on days 9 and 11; this correlated with a high level of transferrin receptor expression on cells harvested on day 9 (Fig 4).

Because human urinary EPO is only partially purified, it is possible that the enhancement of cell proliferation and of surface transferrin receptor expression was due to a contaminating factor in the preparation and not EPO. To address this question directly, cells from individual patients were cultured in the presence of both urinary and recombinant EPO. Results of a representative experiment are given in Table 3. The similar effects of both EPO preparations suggest that EPO is the molecule responsible for the augmentation of proliferation and transferrin receptor expression observed in culture.

DISCUSSION

Although investigation of a variety of cell lines has established a relationship between transferrin receptor density and proliferation, little experimental information is available regarding transferrin receptor expression during the growth and differentiation of normal cells. Erythroid maturation is of particular interest because of the large iron requirement for hemoglobin synthesis. We have shown that monoclonal antibodies that recognize transferrin or its surface receptor profoundly decrease the growth of erythroid progenitors at concentrations that have no effect on myeloid colony development.

Burst development is regulated by the combined effects of burst-promoting activity (BPA) and EPO. Early proliferation and burst formation is dependent on BPA and proceeds in the absence of EPO. Erythropoietin is required for later differentiation and for hemoglobinization of maturing bursts. We found that when mononuclear cells initially cultured in the presence of low concentrations of antitransferrin receptor MoAb TFR-A12 and TFR1-2B were removed from methylcellulose and replated at three or five days, the number of BFU-E−derived colonies was markedly reduced, suggesting that transferrin is necessary early in burst development. Other experiments showed that when antibody addition was deferred for as long as one week, proliferation was arrested, and degeneration of the immature colonies occurred. The additional demonstration that cells
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express a high density of surface transferrin receptors well into second week in culture also suggests that transferrin is required during both EPO-dependent and EPO-independent stages of burst maturation. In contrast, cells that were either preincubated in IMDM containing 10 μg/mL of antireceptor antibody or that were removed from cultures containing antibody after a single day and replated yielded normal numbers of BFU-E colonies when washed and plated without antibody. These results suggest that BFU-E have low transferrin receptor expression in vivo that increases in response to culture conditions that promote differentiation of progenitors into mature bursts.

An interesting finding was that although both antireceptor antibodies reduced erythroid colony development at low concentrations, their dose-response effects were somewhat different. As little as 0.5 μg/mL of TFR1-2B completely abrogated burst growth. In contrast, the addition of between 0.1 and 1.0 μg/mL of TFR-A12 decreased erythroid progenitor colony growth in a dose-related fashion. Increasing the concentration of TFR-A12 above 1.0 μg/mL did not further reduce the number of colonies; however, the bursts that were present contained few cells and visibly less hemoglobin. The mechanism by which TFR1-2B and TFR-A12 inhibit cellular proliferation has not been established. These antibodies recognize separate sites on the transferrin receptor that are distinct from the binding site for transferrin. Uptake of transferrin-bound iron by K562 cells is unaffected by either TFR-A12 or TFR1-2B. We have recently shown that fluorescein-labeled TFR1-2B remains bound on the surface of K562 target cells at 37°C; subsequent binding of transferrin to the receptor triggers endocytosis of the antibody-transferrin-receptor complex. Transferrin and TFR1-2B are co-internalized and accumulate in the same perinuclear cluster of vesicles. We have no evidence as yet that TFR1-2B alters recycling of the transferrin receptor to the cell surface or inhibits the release of iron from vesicles, although either mechanism might explain its effects on the growth of BFU-E-derived colonies. The antitransferrin antibody TF-6, which does not bind to cell membranes, produced selective inhibition of erythroid colony growth similar to that observed with TFR-12B and TFR-A12. This finding is important because it suggests that the antireceptor antibodies act specifically by depriving growing cells of transferrin. Our observation that a discrete threshold concentration of TF-6 completely abrogates burst formation is consistent with a previous report that cell lines will undergo maximal proliferation only when a critical amount of transferrin is available to them.

Whether proliferation is triggered by the accumulation of sufficient intracellular iron or is due to some other aspect of the interaction of transferrin with its receptor is unclear. It is attractive to hypothesize that erythroid cells, which need large amounts of iron to synthesize hemoglobin, will only undergo clonal expansion when a critical amount of intracellular iron is present. Recent evidence suggests that intracellular iron levels are crucial in regulating the expression of transferrin receptors on growing cell lines. Taeule and associates investigated the requirement of myeloid leukemia cells for transferrin in serum-free culture medium. Exogenous soluble iron reversed the inhibitory effects of a MoAb against the transferrin receptor on cell growth, and increased intracellular ferritin concentrations were noted in cells that were able to grow in the presence of antibody. We and others have shown that erythroblasts plucked from bursts have a higher density of surface transferrin receptors than cells from CFU-c-derived colonies; yet we found that erythroid colony formation is inhibited by lower concentrations of monoclonal antibodies that bind transferrin or its receptor. The observation that the proliferation and differentiation of erythroid progenitors, which possess a higher density of transferrin receptors than myeloid cells, is inhibited by much lower concentrations of MoAb suggests that development of erythroid bursts from BFU-E requires that many more transferrin molecules be bound to the receptor than are necessary for CFU-c growth. Our results are consistent with the hypothesis that erythroid progenitors must accumulate relatively high intracellular levels of iron to carry out the differentiation program to mature bursts and that this accounts for their sensitivity to antibodies that recognize transferrin or its surface receptor.

We have shown that EPO increases proliferation and transferrin receptor expression by cultured mononuclear cells and that this coincides with morphologic maturation of erythroid bursts. Our findings in studies of normal human progenitors are consistent with a preliminary report that observed that EPO increased both the synthesis of transferrin receptors and their expression on Friend virus-transformed cells. It is likely that the augmentation of iron uptake induced by EPO that has previously been observed in mouse erythroleukemia (Friend) cells is modulated through increased expression of surface transferrin receptors.

Erythroid progenitors both express a high density of surface transferrin receptors and are extremely sensitive to inhibition by monoclonal antibodies against transferrin or its receptor. Our findings suggest that transferrin plays a major role in determining the proliferation and differentiation of BFU-E colonies.
regulatory role in the process by which BFU-E differentiate into bursts. Further study will be required to characterize the relationship between intracellular iron levels and burst growth and to precisely define the effects of EPO on the expression and function of transferrin receptors present on normal erythroid cells.

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