RAPID COMMUNICATION

Real-Time Method for Determining the Colony-Forming Cell Content of Human Hematopoietic Cell Cultures

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Abstract: Glucose and lactate metabolic rates were evaluated for cultures of cord blood (CB) mononuclear cell (MNC), peripheral blood (PB) MNC, and PB CD34$^+$ cell cultures carried out in spinner flasks and in T-flasks in both serum-containing and serum-free media. Specific glucose uptake rates (q_{gluc}, in micromoles per cell per hour) and lactate generation rates (q_{lac}) correlated with the percentage of colony-forming cells (CFC) present in the culture for a broad range of culture conditions. Specifically, the time of maximum CFC percentage in each culture coincided with the time of maximum q_{gluc} and q_{lac} in cultures with different seeding densities and cytokine combinations. A two-population model ($Q_{lac} = \alpha[CFC] + \beta([TC] - [CFC])$, where [TC] is total cell concentration; Q_{lac} is volumetric lactate production rate in micromoles per milliliter per hour; α is q_{lac} for an average CFC; and β is q_{lac} for an average non-CFC) was developed to describe lactate production. The model described lactate production well for cultures carried out in both T-flasks and spinner flasks and inoculated with either PB or CB MNC or PB CD34$^+$ cells. The values for α and β that were derived from the model varied with both the inoculum density and the cytokine combination. However, preliminary results indicate that cultures carried out under the same conditions from different samples with similar initial CD34$^+$ cell content have similar values for α and β. These findings suggest that it should be possible to use lactate production data to predict the harvest time that corresponds to the maximum number of CFC in culture. The ability to harvest ex vivo hematopoietic cultures for transplantation when CFC are at a maximum has the potential to speed the rate at which immunocompromised patients recover. © 1997 John Wiley & Sons, Inc. *Biotechnol Bioeng* **55:** 693–700, 1997.
Keywords: glucose; lactate; real-time determination; hematopoietic cell culture; colony-forming cells

INTRODUCTION

The culture of hematopoietic cells for transplantation therapies is a rapidly growing area of biotechnology and experimental hematology. As evidenced by recent clinical trials (Brugger et al., 1995), ex vivo expanded hematopoietic cells offer great promise for the reconstitution of in vivo hematopoiesis in immunocompromised patients who have undergone chemotherapy. Other potential applications for ex vivo expansion include production of cycling stem and progenitor cells for gene therapy, expansion of dendritic cells for immunotherapy, and production of red blood cells and platelets for transfusions (McAdams et al., 1996b).

The heterogeneous cell population contained in a hematopoietic culture is always changing as a result of the delicate balance between proliferation of certain cell types, their differentiation into other cell types, and the death of various cell populations. The lifespan of cells in culture is likely to depend on cytokine stimulation as well as on a number of physicochemical parameters, such as pH, dissolved oxygen, and nutrient and metabolite concentrations (McAdams et al., 1996a).

Current enumeration techniques for hematopoietic cultures do not provide real-time analysis of the changing populations. Complete evaluation of the performance of hematopoietic cultures requires the use of assays with long duration such as the 2-week methylcellulose assay to detect progenitor or colony-forming cells (CFC), including colony-forming units–granulocyte/monocyte (CFU-GM) and burst-forming units–erythroid (BFU-E), and the 7-week assay for the very primitive long-term culture-initiating cells (LTC-IC). In this regard, the cell requirements for successful engraftment are often expressed in terms of the number of CFC transplanted [e.g., 2×10^5 CFU-GM/kg body weight (Bender et al., 1992)]. In contrast to the long assay times, the time period available to determine when to harvest ex vivo cultures for transplantation therapies is most likely on the order of hours. Currently, only flow cytometry offers this speed of analysis. Flow cytometry can be utilized to quantify cells bearing antigens such as CD34 (primitive progenitors), CD15 and CD11b (granulocyte and monocyte postprogenitors), and gly A (maturing erythrocytes). Even so, sample preparation and measurement, along with data analysis, requires 2–3 h. Furthermore, when cells bearing

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the antigen of interest are present at a low concentration, as is often the case for CFC, accurate quantitation may be difficult. Because of the difficulty in determining when CFU-GM, BFU-E, or other cell populations of interest reach a maximum level, culture endpoints have generally been chosen based on a retrospective analysis of the culture duration that usually yields an acceptable product.

Nutrient consumption and by-product accumulation rates are parameters that can be readily measured in real time. These rates are frequently employed for the control of more traditional cell cultures (for vaccine and protein production) but have been largely overlooked in the evaluation of hematopoietic cultures.

Normal and leukemic human blood cells depend heavily upon glycolysis as their source of energy (Beck, 1958; Beck and Valentine, 1952a,b), and the rates of glucose consumption and lactate production can be altered by external stimuli such as growth factors. Growth factor–stimulated increases in glucose utilization have been demonstrated in cultures of murine macrophages (Hamilton et al., 1986) and multipotential hematopoietic cell lines (Spielholz et al., 1995; Whetton et al., 1984, 1986). Other stimuli that increase the growth rate also affect the glucose uptake rate. Human lymphocytes stimulated to undergo blastogenesis by incubation with phytohemagglutinin (PHA) exhibit increased glucose utilization and lactate production and increased levels of glycolytic pathway enzymes (Hedeskov, 1968; Kester et al., 1977; Rogers et al., 1980). The findings discussed above for stimulated hematopoietic cells are consistent with those for rapidly dividing cells in general, which are known to exhibit rates of glucose consumption and lactate production that are elevated over those of more slowly growing cells (Hume and Weidemann, 1979; Lanks and Li, 1988; Newsholme et al., 1985).

We therefore hypothesized that in a hematopoietic culture containing cells with varying proliferative potential and rates of growth, glucose utilization and lactate production rates would vary with changes in cell type as proliferation and differentiation occurred. We discovered that glucose consumption and lactate generation rates correlate with the progenitor-cell content present at any time in the culture. Our results suggest that real-time measurement of these metabolic indicators may be used to predict optimal harvest times for ex vivo cultures for transplantation applications.

MATERIALS AND METHODS

Media and Reagents

Media

Human long-term medium (HLTM), which was used as the standard serum-containing medium, consists of McCoy's 5A basal medium (Sigma, St. Louis, MO), 12.5% heat-inactivated horse serum (Sigma), 12.5% fetal bovine serum (Hyclone, Logan, UT), 1 m*M* sodium pyruvate (Sigma), 1% modified Eagle's medium (MEM) vitamin solution (Irvine Scientific, Irvine, CA), 1% MEM amino acid solution (Irvine Scientific), 1% MEM nonessential amino acid solution (Irvine Scientific), 10^{-4} *M* monothioglycerol (Sigma), 2 m*M* L-glutamine (Sigma), and 50 μg/mL gentamycin sulfate (Gibco, Grand Island, NY). XVIVO-20 (BioWhittaker, Walkersville, MD) was used as the standard serum-deprived medium.

Cytokines

All cytokines used were purified recombinant human factors. Interleukin-3 (IL-3, Novartis, East Hanover, NJ) was used at 5 ng/mL, IL-6 (Novartis) at 10 ng/mL, stem cell factor (SCF, Amgen, Thousand Oaks, CA) at 50 ng/mL, Flt3 ligand (Flt3-1, Immunex, Seattle, WA) at 50 ng/mL, granulocyte-colony-stimulating factor (G-CSF, Amgen) at 1.5 ng/mL, granulocyte-macrophage-CSF (GM-CSF, Immunex) at 2 ng/mL, and erythropoietin (Epo, Amgen) at 28 ng/mL.

Cells and Cell Separation Procedures

Patient samples (Response Oncology, Memphis, TN) of peripheral blood cells (PB MNC) were collected after informed consent under protocols approved by the respective institutional review boards. Apheresis products were collected from cancer patients following stem cell mobilization regimens consisting of treatment with G-CSF with or without chemotherapy. The samples were used as received; density gradient separation of the mononuclear cell (MNC) fraction was not required due to minimal erythrocyte content. Umbilical cord blood (CB) samples were provided by Northwestern University Memorial Hospital (Chicago, IL). CB MNC were isolated from the whole sample by density gradient separation on Histopaque (1.077 g/mL, Sigma). Positive selection of CD34 antigen-bearing cells (CFC are contained within the $CD34^+$ cell population) was accomplished by utilizing MiniMACS (Miltenyi Biotech, Sunnyvale, CA) magnetic separation columns following the directions of the manufacturer. The number of nucleated cells was determined on a Coulter Counter Multisizer (Coulter Electronics, Hialeah, FL) after cetrimide (Sigma) treatment to lyse the cells and release the nuclei. The error associated with the preparation and measurement of cell density was estimated to be ±5%.

Methylcellulose Colony Assays

The numbers of granulocyte, monocyte/macrophage, erythroid, and mixed-lineage progenitor cells were determined using a methylcellulose colony assay as described previously (Koller et al., 1992) with slight modifications. The 1.1% methylcellulose medium was supplemented with IL-3, IL-6, SCF, GM-CSF, G-CSF, and Epo at the concentrations listed for liquid culture, with the exception of Epo, which was added at a concentration of 83 ng/mL. Cultures were

plated at seeding densities ranging from 2000 to 15,000 cells/mL for fresh and cultured MNC and from 500 to 10,000 cells/mL for cultures initiated with $CD34^+$ cells. The inoculum density for methylcellulose culture was determined by both the degree of total cell expansion in the culture and the day of culture. In our culture systems, the cloning efficiency drops as the total cell expansion rises. We therefore increased the methylcellulose plating density as the culture expanded in an effort to maintain a total of 100–300 CFC per dish. By so doing, we avoid the effects of either overplating or underplating the methylcellulose culture that occur when a fixed seeding density is used at all time points. The methylcellulose cultures were incubated for 14 days in a humidified atmosphere of 5% O_2 and 5% CO_2 (balance N_2). At the end of the incubation period, colonies of 50 or more cells were enumerated as either CFU-GM (including CFU-G and CFU-M), BFU-E, or CFU-Mix through inspection on a dark-field stereomicroscope (Zeiss, Batavia, IL). The majority of the cultures did not contain detectable numbers of CFU-Mix, so that we neglected CFU-Mix in our analysis. The error associated with enumerating CFC was estimated using Poisson statistics.

Stirred Hematopoietic Culture

Stirred cultures were carried out in 100-mL spinner flasks (Bellco model 1967 with model 1965 agitator assembly) with an agitation rate of 30 RPM (Collins et al., 1996). The spinners were not siliconized prior to use, as this was found to be unnecessary. The cultures were fed every 2 days, beginning at day 4, by pipette removal of one-half of the cell suspension, centrifugation at 300*g* for 10 min, removal of the spent medium, and return of the cells with fresh equilibrated medium to the spinner flask. The spinner flasks were maintained within a humidified incubator at 5% O_2 and 5% CO_2 (balance N_2). Samples containing cells and medium were removed by pipette, and the number of nucleated cells enumerated using a Coulter counter. Medium supernatant samples were frozen at –20°C and retained for metabolite analysis.

Static Hematopoietic Culture

Static cultures were carried out in T-75 flasks (Falcon, Lincoln Park, NJ) for MNC culture or T-25 flasks (Falcon) for $CD34^+$ cell cultures. Static cultures were maintained in the same manner as stirred cultures.

Metabolic Assays and Calculations

Medium supernatant samples were thawed and subsequently centrifuged for 10 min at 14,000 RPM using an Eppendorf model 5415C centrifuge to remove any particulates that could potentially foul the membranes of the YSI model 2700 glucose/lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). The analyzer was calibrated after every six samples to enhance the accuracy of the assays. The manufacturer's stated assay precision is ±2%, while the linearity is ±2% between 0 and 13.9 mmol and ±5% between 13.9 and 138.9 mmol for glucose and ±2% between 0 and 5.6 mmol and ±5% between 5.6 and 29.1 mmol for lactate.

Volumetric glucose consumption and lactate generation rates (Q, in micromoles per milliliter per hour) were calculated using the second-order central slope method, as follows. The total glucose consumed or lactate generated was calculated for each time point. For any time point (t_i), the first-order forward (f, from time t_i to t_{i+1}) and backward (b, from time t_{i-1} to t_i) slopes of the total consumption or generation curve were calculated by dividing the point-to-point metabolite consumption or generation differences by the point-to-point differences in time. The volumetric rate at time t_i was then calculated by taking a time–distance weighted average of these two slopes:

$$Q_i = \frac{(t_{i+1} - t_i)\,(\text{slope}_b) + (t_i - t_{i-1})\,(\text{slope}_f)}{(t_{i+1} - t_{i-1})} \tag{1}$$

Specific metabolic rates (q, in micromoles per cell per hour) at any time t_i were obtained by dividing the volumetric rate by the nucleated cell density X_i (cells per milliliter) at time t_i:

$$q_i = \frac{Q_i}{X_i} \tag{2}$$

The nucleated cell density does not account for enucleated red blood cells (RBC, final stage erythroid cells do not have a nucleus) which may be present in the culture. We did not account for this enucleated population because the small number of RBC present at inoculation are typically no longer detectable by day 3 and we generally do not observe the formation of enucleated RBC in our cultures (as determined by phenotypic examination).

RESULTS AND DISCUSSION

Correlation of Culture CFC Content with Specific Metabolic Rates

In examining the specific glucose consumption rate (q_{gluc}) and lactate generation rate (q_{lac}) for cultures carried out in spinner flasks, we observed that both q_{gluc} and q_{lac} increase from time zero until a maximum is attained. After that time, both q_{gluc} and q_{lac} fall until they reach a minimum value that is maintained until the end of the culture. A similar decrease (after reaching a maximum) in the fraction of cells in a culture that are CFC (%CFC) suggested a relationship between %CFC and q_{gluc} (or q_{lac}). When q_{gluc}, q_{lac}, and %CFC are plotted vs. time on the same graph, they rise and fall simultaneously (Fig. 1), with the maximum q_{gluc} or q_{lac} observed when the percentage of CFC in culture is the greatest. This suggests that rapidly proliferating CFC have a much greater metabolic demand than more mature cells.

In all experiments conducted, similar trends were ob-

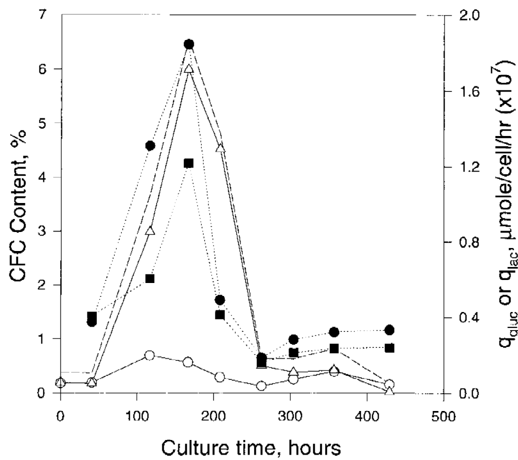

Figure 1. Time profile for q_{gluc} (■), q_{lac} (●), and the percentage of cells that are CFU-GM (○), BFU-E (△), and total CFC (CFU-GM + BFU-E) (– – –) in a spinner flask. The CB MNC culture was carried out in XVIVO-20 with IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo at an inoculum density (ID) of 3.8×10^5 cells/mL.

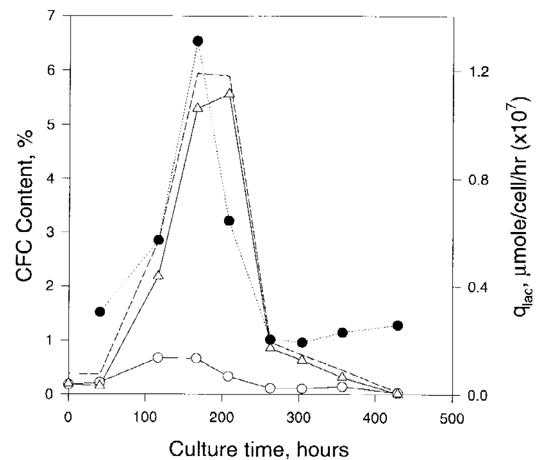

Figure 2. Time profile for q_{lac} and the percentage of cells that are CFU-GM, BFU-E and total CFC (CFU-GM + BFU-E) in a T-flask. The sample and culture conditions used and the symbols are the same as in Figure 1.

served for glucose consumption and lactate production rates. Lactate data generally displayed less scatter than did glucose data. This was especially true early in cultures inoculated at low cell densities where the error in the glucose assay was of the same order of magnitude as the amount of glucose consumed. For simplicity, we hereafter limit our discussion to lactate production rates.

The data shown in Figure 1 are for a CB MNC spinner flask culture conducted using XVIVO-20 with IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo at an inoculum density (ID) of 3.85×10^5 cells/mL. However, the relationship between %CFC and q_{lac} is not dependent upon a particular cell source, culture system, medium type, ID, or cytokine combination. To date, we have observed this relationship in more than 10 CB MNC, 47 PB MNC, and 18 PB $CD34^+$ cell cultures carried out under a variety of conditions. For example, the correlation between q_{lac} and %CFC shown in Figure 1 was also evident in a parallel T-flask culture (Fig. 2). For a given cytokine combination, cultures initiated at different ID from the same PB MNC sample exhibited the same interdependence of q_{lac} with %CFC (Fig. 3). The coincidence of q_{lac} and %CFC was also maintained for different cytokine combinations in cultures initiated at the same ID from the same PB MNC sample (Figs. 4 and 5). The correlation was evident in both serum-containing (Figs. 3 and 6 and results not shown) and serum-free (Figs. 1, 2, 4, and 5 and results not shown) culture media. The shape of the q_{lac} profile is not always the same from culture to culture, but the time of maximum q_{lac} still corresponds to the time of maximum CFC content. The coincidence of maxima in q_{lac} and %CFC also extended to cultures that exhibited local minima and maxima (Figs. 3 and 4B), although the majority of our cultures did not realize increases in %CFC once the decline from maximum %CFC had begun.

The maximum CFC content observed in MNC cultures is typically on the order of 10%. We wanted to determine whether the coincidence of maxima for q_{lac} and %CFC would hold true in cultures with higher CFC content, such as those inoculated with $CD34^+$ cells. In $CD34^+$ cell cultures, the %CFC achieved was much greater (as high as 40%), and the time to maximum %CFC was shorter than in

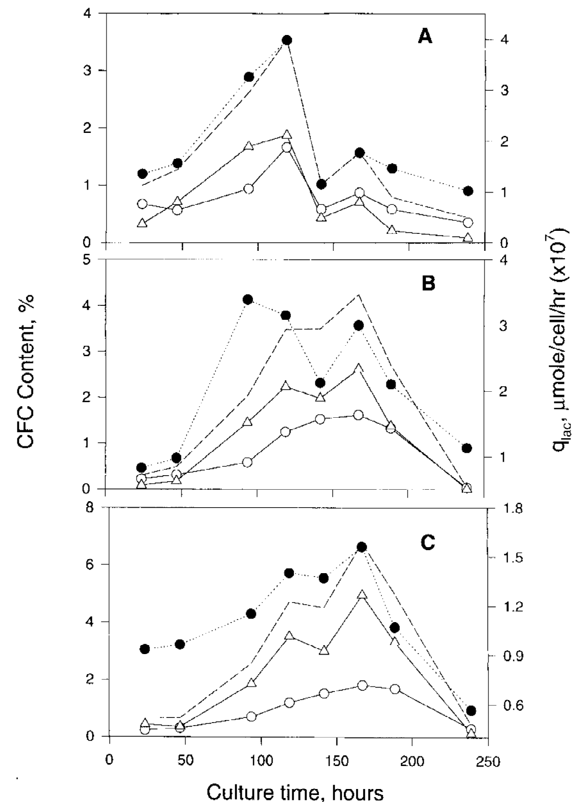

Figure 3. Time profiles for q_{lac} and the percentage of cells that are CFU-GM, BFU-E, and total CFC (CFU-GM + BFU-E) in spinner flasks for a single PB MNC sample cultured at an ID of (A) 160,000 cells/mL, (B) 750,000 cells/mL, and (C) 1,260,000 cells/mL. All cultures were carried out in HLTM with IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo. The symbols are the same as in Figure 1.

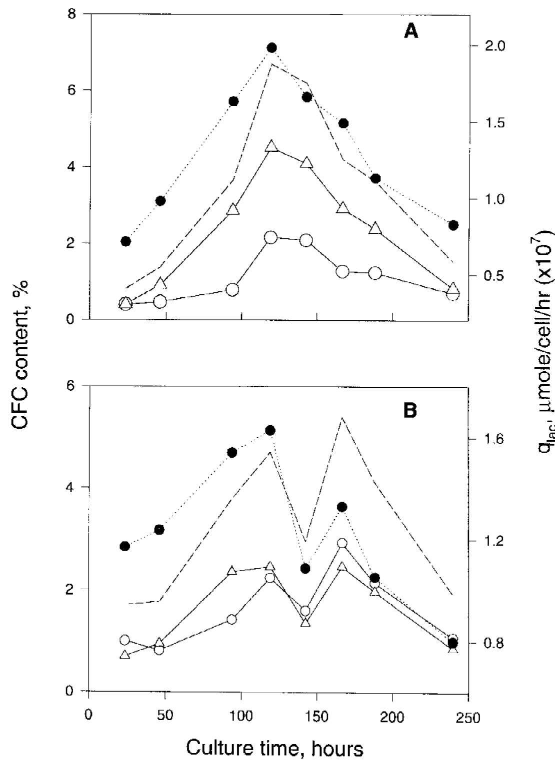

Figure 4. Time profiles for q_{lac} and the percentage of cells that are CFU-GM, BFU-E, and total CFC (CFU-GM + BFU-E) in spinner flasks for a single PB MNC sample cultured at an ID of 1,260,000 cells/mL in XVIVO-20 with IL-3, IL-6, SCF, G-CSF, and either (A) GM-CSF and Epo or (B) Flt3-1. The symbols are the same as in Figure 1.

MNC cultures. Despite the differences in initial culture population, maximum CFC content attained, and growth kinetics, the %CFC content was reasonably paralleled by q_{lac} in CD34$^+$ cell cultures with three different ID (Fig. 6). However, it should be noted that the point corresponding to the first calculated q_{lac} value for the three cultures shown in Figure 6, as well as for most other CD34$^+$ cell cultures, was well below that expected for a culture with such a high %CFC. We attribute the low q_{lac} to the fact that primitive hematopoietic cells are predominantly in quiescence at the onset of culture (Gore et al., 1995; Traycoff et al., 1994) and would therefore not be expected to demonstrate the high rate of lactate generation associated with rapid proliferation. This period of quiescence applies to MNC cultures as well, but the %CFC present at the beginning of a MNC culture is much lower than that for a CD34$^+$ cell culture.

Modeling Cell Metabolism

An exact model of total lactate production in our cultures would consider each distinct cell type, such that

$$\frac{\Delta\,(\text{lactate})}{\Delta\,(\text{time})} = \sum_i (q_{\text{lac}i} \times n_i) \qquad (3)$$

where i represents each individual cell type and n_i the number of cells of type i. However, the large number of hematopoietic cell types makes this model unwieldy. The dramatic decrease in q_{lac} during the differentiation from CFC to postprogenitor cells (e.g., see Fig. 5A at 120 h) suggests that a two-population (CFC and other cells) model may provide an adequate description of lactate production in our cultures, such that

$$Q_{\text{lac}} = \alpha[\text{CFC}] + \beta([\text{TC}] - [\text{CFC}]) \qquad (4)$$

where α is the q_{lac} value for a CFC, β is the q_{lac} value for a non-CFC, [TC] is the concentration of total nucleated cells in the culture, and [CFC] is the concentration of total CFC in the culture. Equation (4) can be normalized by dividing both sides by [TC]. Upon rearrangement, the following relationship is obtained:

$$\frac{Q_{\text{lac}}}{[\text{TC}]} = (\alpha - \beta)\frac{[\text{CFC}]}{[\text{TC}]} + \beta \qquad (5)$$

or

$$q_{\text{lac}} = (\alpha - \beta)\frac{(\%\text{CFC})}{100} + \beta \qquad (6)$$

If this two-population model adequately describes the data, a plot of q_{lac} versus %CFC in a culture will yield a straight line with the y intercept giving q_{lac} for a non-CFC

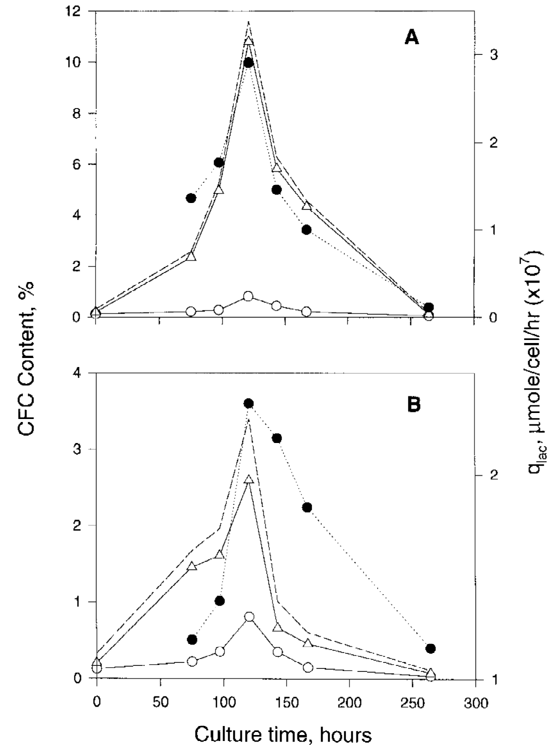

Figure 5. Time profiles for q_{lac} and the percentage of cells that are CFU-GM, BFU-E, and total CFC (CFU-GM + BFU-E) in spinner flasks for a single PB MNC sample cultured at an ID of 800,000 cells/mL in XVIVO-20 with IL-3, IL-6, SCF, and either (A) Epo or (B) G-CSF. The symbols are the same as in Figure 1.

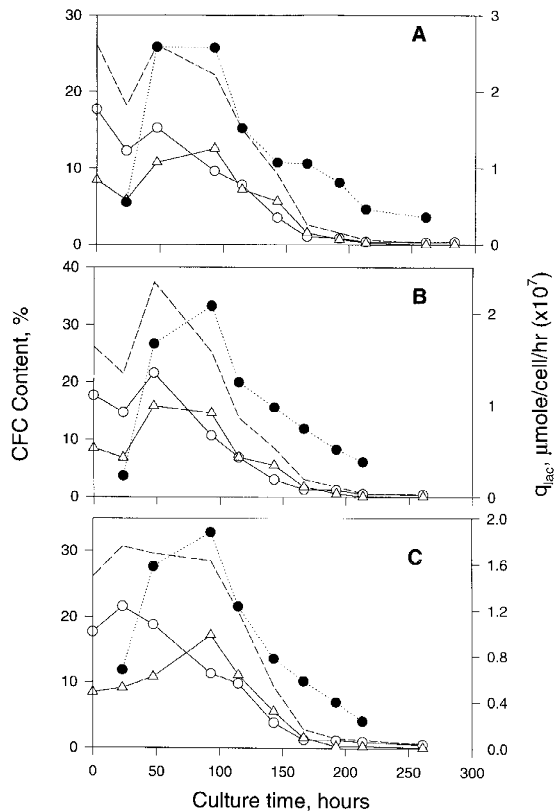

Figure 6. Time profiles for q_{lac} and the percentage of cells that are CFU-GM, BFU-E, and total CFC (CFU-GM + BFU-E) in T-flasks for a single PB CD34$^+$ cell sample cultured at an ID of (A) 33,000 cells/mL, (B) 82,000 cells/mL, and (C) 125,000 cells/mL. The cultures were conducted in HLTM with IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo. The symbols are the same as in Figure 1.

and the slope yielding the difference between q_{lac} for a CFC and that for a non-CFC.

Figure 7 shows the linear relationship between q_{lac} and %CFC for a CB MNC culture in HLTM with IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo. The line though the data points was generated by linear regression. In Figure 7, the y intercept β (q_{lac} for a non-CFC) is 2.5×10^{-8} µmol/cell/h. The calculated α for the data in Figure 7 is 5.3×10^{-6} µmol/cell/h. Since α is approximately 200-fold greater than β, this substantiates our hypothesis that the average CFC has a much greater q_{lac} than does a more differentiated cell.

Regression analysis (Fig. 8) was also performed on the data previously presented in time-course form in Figure 3. As before, a reasonable straight-line relationship was obtained between q_{lac} and %CFC. Again, the calculated α is much greater than β for each plot. The degree of correlation varied for the different cultures, but in most cases points that deviate from the regression line are explained by the errors associated with the %CFC and q_{lac} calculations.

As mentioned above, hematopoietic progenitors are typically quiescent at the onset of culture. Quiescent CFC would not be expected to exhibit high lactate production rates. Thus, our proposed model would be expected to only describe lactate production for cells that have exited quies-

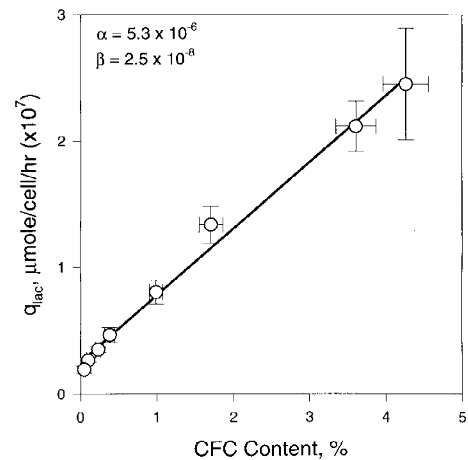

Figure 7. q_{lac} vs. %CFC for a CB MNC culture carried out in a spinner flask at an ID of 250,000 cells/mL in HLTM plus IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo. The correlation coefficient for the line is 0.99. The error bars indicate the mean ± one standard deviation. Standard deviations were determined via propagation of the errors in cell density (5%), lactate concentration (2% or 5%; see Methods), and CFC ($\sqrt{N}/N$, where N is the total number of colonies counted) through the calculations for q_{lac} and %CFC.

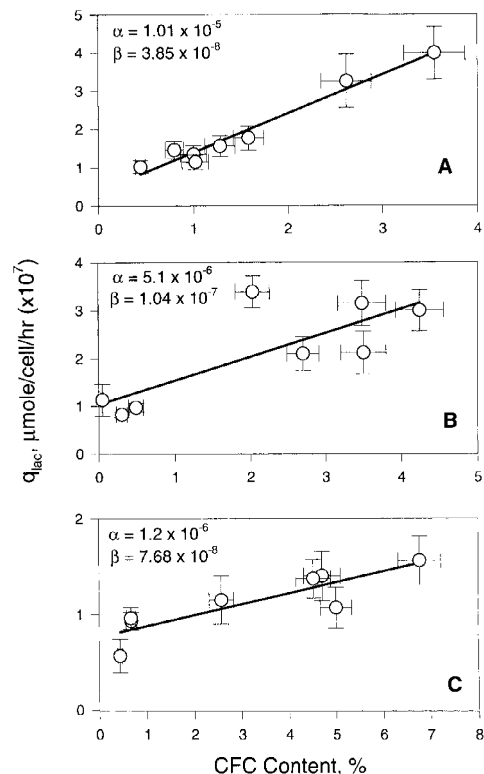

Figure 8. q_{lac} vs. %CFC in spinner flasks for a single PB MNC sample cultured at an ID of (A) 160,000 cells/mL, (B) 750,000 cells/mL, and (C) 1,260,000 cells/mL. The cultures were carried out in HLTM plus IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo. The data are the same as those shown in time-course format in Figure 3. The correlation coefficients for the lines are (A) 0.96, (B) 0.63, and (C) 0.74. The error bars indicate the mean ± one standard deviation and were determined as described for Figure 7.

cence. This is not a major limitation because cells typically leave quiescence within 24–48 h of culture (Traycoff et al., 1994). Indeed, linear regressions of the Figure 6 data for $CD34^+$ cell cultures describe the relationship between %CFC and q_{lac} reasonably well after the first time point in the culture, which is associated with the lag phrase, is removed (Fig. 9).

Our proposed model could be used to predict the total CFC content if all cultures had the same values for α and β. However, these parameters vary with culture conditions such as cytokine combination, inoculum density, and cell type. It is more likely that the same α and β values will be obtained for samples with similar $CD34^+$ cell content cultured under identical conditions. Indeed, when three different PB MNC samples with similar day-zero $CD34^+$ cell content were cultured under identical conditions, the values for α and β were similar enough that the data could be pooled into one correlation, as shown in Figure 10.

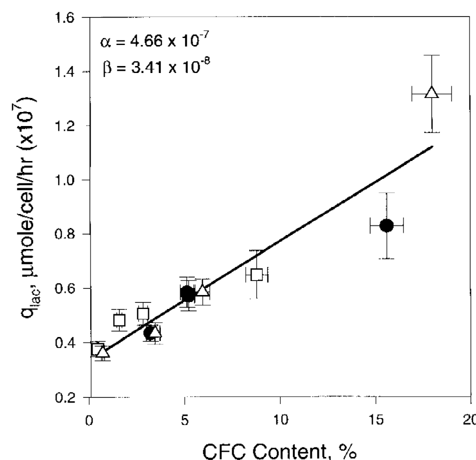

Figure 10. q_{lac} vs. %CFC for three PB MNC cultures carried out in spinner flasks with HLTM plus IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo at an ID of 1.2×10^6 cells/mL. The day-zero $CD34^+$ cell content of the samples were (●) 6.6%, (□) 6.3%, and (△) 5.8%. The correlation coefficient for the line is 0.88. The error bars indicate the mean ± one standard deviation and were determined as described for Figure 7.

CONCLUSIONS

Our data demonstrate that changes in the CFC content of a culture can be followed by monitoring q_{lac}. A correlation between q_{lac} and %CFC was evidenced over a wide variety of conditions, including spinner flask (Figs. 1 and 3–5) and T-flask (Figs. 2 and 6) cultures with MNC (Figs. 1–5) and $CD34^+$ cells (Fig. 6) in serum-containing media (Figs. 3 and 6) and serum-free media (Figs. 1, 2, 4, and 5) with different cytokines (Figs. 4 and 5) and inoculum densities (Figs. 3 and 6).

The coincidence of maximum %CFC with maximum q_{lac} may be useful in deciding when to manipulate a hematopoietic culture. For example, for some applications of gene therapy using ex vivo expanded cells, it might be best to initiate gene transfer when the progenitor cell content is highest (i.e., the time of maximum %CFC and q_{lac}). However, if the expanded cells are to be used directly for transplantation, it will likely be more beneficial to harvest when the total content of CFC (or other desired cell types) reaches a maximum. Any particular clinical protocol is likely to be restricted to a single cell type (PB or CB; MNC or $CD34^+$ cells), culture system, cytokine combination, and inoculum density. The correlation shown in Figure 10 suggests that it will be possible to identify unique α and β values for these cultures, or at least distinct α and β values for different ranges of $CD34^+$ cell content in MNC cultures. Since the $CD34^+$ cell content is routinely measured for hematopoietic cell harvests, this means that the appropriate α and β values would be available at the beginning of each culture. In this event, it should be possible to predict the total CFC content [from Equation (4)] as well as the %CFC [from Equation (6)] at any time point in the culture, thereby realizing real-time determination of CFC content in culture.

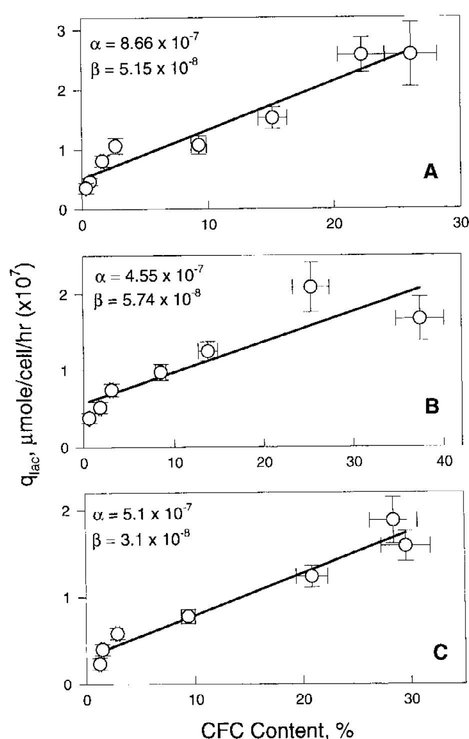

Figure 9. q_{lac} vs. %CFC in T-flasks for a single PB $CD34^+$ cell sample cultured at an ID of (A) 33,000 cells/mL, (B) 82,000 cells/mL, and (C) 125,000 cells/mL. The cultures were carried out in HLTM plus IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo. The data are the same as those shown in time-course format in Figure 6, except that the first time point has been deleted from each culture (see text). The correlation coefficients for the lines are (A) 0.94, (B) 0.79, and (C) 0.95. The error bars indicate the mean ± one standard deviation and were determined as described for Figure 7.

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