

Characterization of Hematopoietic Cell Expansion, Oxygen Uptake, and Glycolysis in a Controlled, Stirred-Tank Bioreactor System

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Cultures of umbilical cord blood and mobilized peripheral blood mononuclear cells were carried out in a stirred bioreactor with pH and dissolved oxygen control. Expansion of total cells and colony-forming units granulocyte-macrophage was greatly enhanced by the use of a cell-dilution feeding protocol (as compared to a cell-retention feeding protocol). The specific oxygen consumption rate (q_{O_2}) for these cultures ranged from 1.7×10^{-8} to 1.2×10^{-7} $\mu\text{mol}/(\text{cell}\cdot\text{h})$. The maximum in q_{O_2} for each culture closely corresponded with the maximum percentage of progenitor or colony-forming cells (CFCs) present in the culture. The maximum q_{O_2} values are slightly less than those reported for hybridomas, while the lowest q_{O_2} values are somewhat greater than those reported for mature granulocytes. Examination of the ratio of lactate production to oxygen consumption in these cultures suggests that post-progenitor cells of the granulomonocytic lineage obtain a greater portion of their energy from glycolysis than do CFCs. The different metabolic profiles of CFCs and more mature cells suggest that monitoring the uptake or production of oxygen, lactate, and other metabolites will allow estimation of the content of several cell types in culture.

Introduction

Recent clinical trials (Williams et al., 1996; Bertolini et al., 1997) have demonstrated that ex vivo expanded hematopoietic cells offer great promise in reconstituting in vivo hematopoiesis in patients who have undergone intensive chemotherapy. Thus, it is likely that the demand for ex vivo expanded hematopoietic cells for transplantation in the treatment of cancer will increase dramatically. It is therefore necessary to develop clinical-scale culture systems that both provide expansion of desired cell types and satisfy FDA requirements (U.S. Food and Drug Administration, 1997). Recently, we described the successful application of spinner flask culture for hematopoietic cells from a variety of sources in both serum-containing and serum-free media (Collins et al., 1998). Spinner flask systems have also been used to culture bone marrow (BM) mononuclear cells (MNCs) (Zandstra et al., 1994; Sardonini and Wu, 1993). Although peripheral blood (PB) MNC-derived natural killer (NK) cells have been cultured in a stirred bioreactor (Pierson et al., 1996), controlled, stirred-tank bioreactor systems have not yet been reported for the culture and characterization of myeloid-lineage hematopoietic cells.

A well-controlled, closed, and reproducible culture environment, such as that offered by stirred bioreactors, will undoubtedly prove advantageous for clinical applications, especially considering the scale involved for clinical cultures. The culture volume employed for recent clinical trials averaged about 5 L (Zimmerman et al., 1995; Williams et al., 1996). Fifty T-150 flasks each containing

100 mL of culture medium or 20 gas-permeable 300-cm² culture bags each containing 250 mL would be necessary to accommodate this volume. These phase I clinical trials were conducted to determine the safety of infusing expanded cells. As trials continue, greater numbers of cells will undoubtedly be transfused in an effort to increase the efficacy of expansion protocols.

While bone marrow was the traditional source of hematopoietic cells for transplantation therapies, other blood cell sources are becoming more popular. Mobilized PB progenitor cell transplants have proved effective, and it is likely that mobilized PB MNC (hereafter just PB MNC) will replace BM MNC as the preferred source of hematopoietic cells for transplantation (Korbling and Champlin, 1996). Umbilical cord blood (CB) is both readily available and easily collected. CB stem cells are thought to be more immature than those found in adults. This attribute makes CB stem cells a potential target for the correction of genetic blood diseases (Clapp and Williams, 1995). We demonstrate that both CB and PB MNC can be successfully cultured in a stirred bioreactor configuration.

Traditional hematopoietic cell cultures also suffer from an inability to fully characterize cell behavior. We have previously demonstrated that glucose and lactate metabolic rates are directly related to the progenitor or colony-forming cell (CFC) content of hematopoietic cultures (Collins et al., 1997). Data regarding oxygen consumption rates in human hematopoietic cultures are scarce, and the published reports have not fully examined the effect of various cell populations on glucose and oxygen metabolism. We therefore examined the effect of different hematopoietic populations (CFCs and more mature cells) on oxygen consumption and on the ratio of glycolytic to oxidative metabolism. We found that the maximum specific oxygen consumption rate (q_{O_2}) corresponds to the maximum in the percentage of CFCs present. The ratio of the specific lactate production rate (q_{lac}) to q_{O_2} increased

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late in the culture, indicating that glycolysis plays a greater role in energy production for more mature cells.

Materials and Methods

Medium. Human long term medium (HLTM), which is based on McCoys 5A medium and contains 12.5% fetal bovine serum (FBS) and 12.5% horse serum (HS), was prepared as previously described (Collins et al., 1997). HLTM was supplemented with purified recombinant human cytokines: 5 ng/mL interleukin-3 (IL-3, Novartis, East Hanover, NJ), 50 ng/mL IL-6 (Novartis), 50 ng/mL stem cell factor (SCF, Amgen, Thousand Oaks, CA), 1.5 ng/mL granulocyte-colony stimulating factor (G-CSF, Amgen), 2 ng/mL granulocyte-macrophage-CSF (GM-CSF, Immunex, Seattle, WA), and 28 ng/mL erythropoietin (Epo, Amgen).

Cells and Cell Separation Procedures. Patient samples (Response Oncology, Memphis, TN) of mobilized 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. Samples in 15-mL polystyrene test tubes containing anticoagulant citrate dextrose were stored and shipped under ambient conditions and used as received within 2–3 days of collection; enrichment of the MNC fraction was not required due to minimal erythrocyte content. CB samples were provided by Northwestern University Memorial Hospital (Chicago, IL). Erythrocytes were depleted from the whole sample by ammonium chloride lysis (Denning-Kendall et al., 1996). All samples were incubated for 2–4 days at $(1.2-2) \times 10^6$ cells/mL in T-150 flasks (Falcon, Lincoln Park, NJ) prior to inoculation in either a T-75 flask or a stirred bioreactor. This preincubation period was designed to acclimate the cells to the culture conditions of the experiment in the absence of potential fluid-mechanical damage. In addition, it allowed us to avoid a lag phase by transferring exponentially growing cells into the bioreactor. The number of nucleated cells was determined on a Coulter Counter Multisizer (Coulter Electronics, Hialeah, FL) after cetrimide (Sigma, St. Louis, MO) treatment to lyse the cells and release the nuclei.

Methylcellulose Colony Assays. The numbers of granulocyte and monocyte/macrophage (collectively CFU-GM) and erythroid (BFU-E) progenitor cells (colony-forming cells, or CFCs) were determined using a methylcellulose colony assay as previously described (Collins et al., 1997).

Culture Conditions. Stirred bioreactor cultures were carried out in a 400-mL B. Braun Biostat Q (B. Braun Biotech USA, Allentown, PA) with an agitation rate of 30 rpm at a working volume of 150–200 mL. The reactor's stainless steel agitator assembly was removed because we have observed detrimental effects of stainless steel on hematopoietic cell proliferation (LaIuppa et al., 1997). We fitted a Bellco (Vineland, NJ) spinner flask model 1965-250 agitator assembly into a compression fitting on the head plate of the reactor. The inside diameter of the Biostat Q is the same as that for the Bellco spinner flask model 1967-100. The agitation setup was therefore identical (except for the longer agitator shaft) to that employed previously ($d_i/D = 0.8$) in our spinner flasks (Collins et al., 1998). The reactor was maintained within a 37 °C incubator and was fitted with dissolved oxygen (DO, Ingold, Wilmington, MA) and pH (Ingold) probes, which were interfaced to a PC via the

Workbench PC program (Omega, Stamford, CT). DO was controlled at 50% of air saturation through headspace addition of humidified O₂, N₂, and air. pH was controlled at 7.33 ± 0.03 through head space addition of humidified CO₂. Static cultures were carried out in T-75 flasks (Falcon, Lincoln Park, NJ) maintained at 37 °C inside a 5% CO₂ (balance air) incubator.

Feeding Protocols. The cultures in experiment 1 were fed every 2 days, beginning at day 4, by pipet removal of one-half of the cell suspension, centrifugation at 300g for 10 min, removal of the spent medium, and return of the cells with fresh equilibrated medium to the culture vessel, thereby maintaining a constant culture volume. This feeding protocol was used in the development of a spinner culture system for PB and CB MNC (Collins et al., 1998) and is designated feeding protocol 1 (FP1). In subsequent experiments, cultures were diluted daily (experiments 3 and 4) or every 2 days (experiment 2) to a density of $(1.5-2) \times 10^6$ cells/mL. Practically, 25–45% of the culture broth (depending on the measured cell density) was removed and the reactor replenished with fresh medium (Feeding Protocol 2, FP2). T-flask controls were fed with a volumetric exchange equivalent to that in the bioreactor. Cell expansion ratios were calculated by determining the total cells that would have been produced in the vessel, assuming that the removed cells expanded in a manner identical to that of the remaining cells. Medium supernatant samples were frozen at –20 °C and retained for metabolite analysis.

Metabolic Assays and Calculations. Medium supernatant samples were thawed and subsequently analyzed on a YSI model 2700 glucose/lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH) as previously described (Collins et al., 1997). Specific glucose and lactate metabolic rates (q , $\mu\text{mol}/(\text{cell}\cdot\text{h})$) at any time t_i were obtained as follows:

$$q_i = \frac{(t_{i+1} - t_i) \left(\frac{Q_b}{X_b} \right) + (t_i - t_{i-1}) \left(\frac{Q_f}{X_f} \right)}{(t_{i+1} - t_{i-1})} \quad (1)$$

where X_b and X_f are the log-mean average cell densities (cells/mL) for the time periods before and after time i , Q_b is the point-to-point volumetric glucose consumption or lactate generation rate (e.g., $\Delta[\text{lactate}]/\Delta t$, $\mu\text{mol}/(\text{mL}\cdot\text{h})$) from time t_{i-1} to t_i , and Q_f is point-to-point rate from time t_i to t_{i+1} . The specific glucose and lactate metabolic rates were calculated in this manner because of the changes in cell density associated with FP2.

The specific oxygen uptake rate (q_{O_2} , $\mu\text{mol}/(\text{cell}\cdot\text{h})$) was determined for experiments 1 and 2 using the steady-state method (Miller et al., 1988) and for experiments 3 and 4 using the dynamic method (Zhou and Hu, 1994). For experiments 3 and 4, q_{O_2} was also calculated using the steady-state method on a number of occasions. We noted good agreement between the two calculation methods. The ratio of the lactate production rate to the oxygen consumption rate ($Y_{\text{lac,ox}}$) was calculated as:

$$Y_{\text{lac,ox}} = \frac{q_{\text{lac}}}{q_{O_2}} \quad (2)$$

Results

Effect of Culture System and Feeding Protocol on Total Cell and Colony-Forming Units Granulocyte-Macrophage (CFU-GM) Expansion. Our first bioreactor experiment utilized FP1; the total cell and

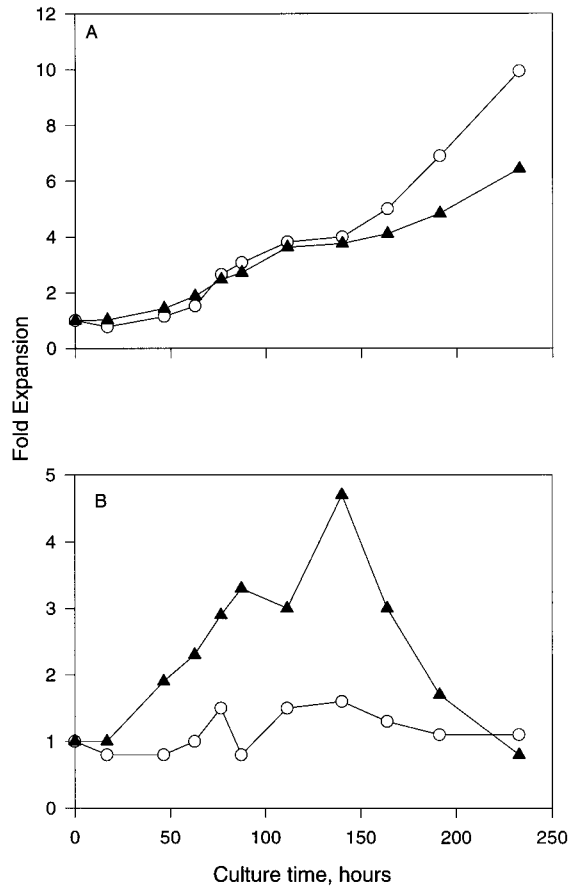


Figure 1. Time profiles for the expansion of (A) total cells and (B) CFU-GM for a single CB MNC sample (experiment 1) cultured in a T-flask (▲) and a bioreactor (○) at an ID of 1.37×10^6 cells/mL. The final culture density was 6.2×10^6 cells/mL in the bioreactor and 8.8×10^6 cells/mL in the T-flask. The cultures were conducted in HLTM with IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo and were fed using FP1—except that the bioreactor was diluted from 5.2×10^6 to 3.2×10^6 cells/mL at 144 h.

CFU-GM expansion ratios for this experiment are shown in Figure 1. The bioreactor and T-flask had comparable total cell expansion profiles until day 6, when the total cell concentration in both cultures appeared to have plateaued. On day 6, in an effort to increase the culture growth rate, the bioreactor cell density was diluted from 5.2×10^6 to 3.2×10^6 cells/mL, while the T-flask cell density (5.1×10^6 cells/mL) was not changed. The bioreactor was fed using FP1 both before and after this single dilution event. After the dilution, the rate of total cell expansion in the bioreactor increased again (as it did in the T-flask, although to a lesser extent) and a separation was evident between the performance of the two vessels.

We hypothesized that a dilution-feeding protocol, such as FP2, would increase the extent of total cell expansion in our cultures. We therefore utilized FP2 for both the T-flask and the bioreactor in subsequent experiments. Figure 2 shows the cell density in a CB MNC experiment (experiment 3) utilizing FP2 throughout the culture period. Through repeated dilution and feeding, cell growth was maintained for an extended period of time in both the reactor and the T-flask. As suspected, FP2 resulted in a much greater expansion of total cells in both vessels (Figure 3) than did FP1 (Figure 1A). If the expansion product is to be infused into a patient for the purpose of reconstituting the hematopoietic system following chemotherapy, CFU-GM cells are likely to be

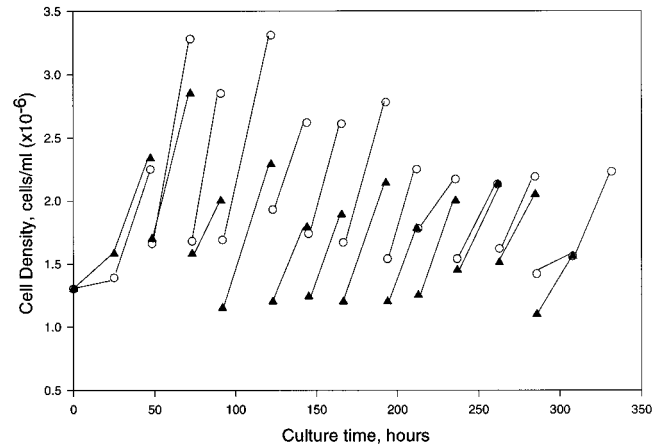


Figure 2. Time profile of the cell density for a single CB MNC sample (experiment 3) cultured in a T-flask (▲) and in a bioreactor (○) at an ID of 1.3×10^6 cells/mL. The cultures were conducted in HLTM with IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo and were fed using the dilution feeding protocol (FP2).

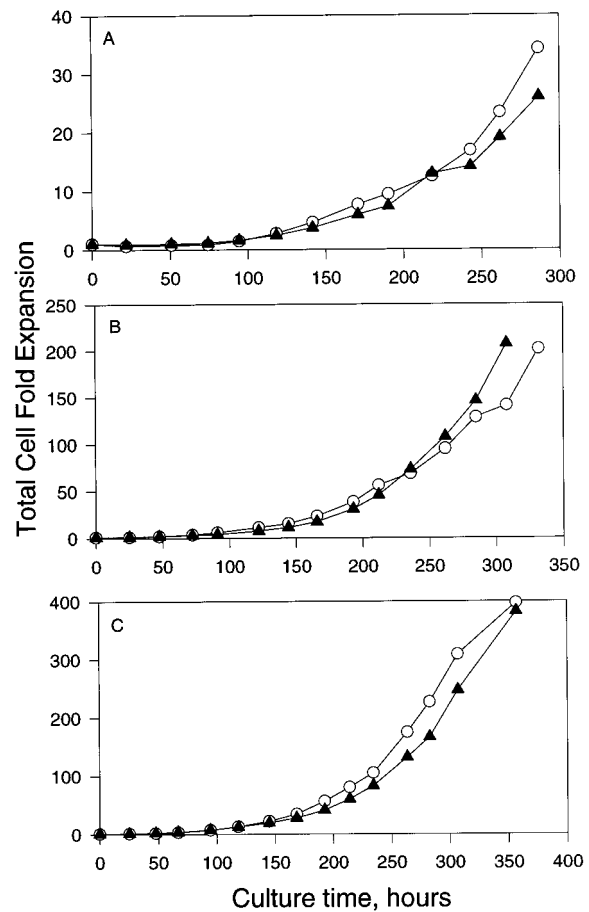


Figure 3. Time profiles for total cell expansion in a T-flask (▲) and a bioreactor (○) using FP2 for (A) experiment 2 (CB MNC, ID = 1.72×10^6 cells/mL), (B) experiment 3 (CB MNC, ID = 1.3×10^6 cells/mL), and (C) experiment 4 (PB MNC, ID = 2.24×10^5 cells/mL).

particularly important, since they give rise to critical infection-fighting granulocytes. CFU-GM expansion in FP2-fed systems (Figure 4) was much greater than that observed in either the bioreactor or T-flask FP1-fed cultures (Figure 1B).

Metabolic Rates in the Bioreactor. Figure 5 shows specific lactate (q_{lac}) and oxygen (q_{O_2}) metabolic rates, along with culture CFC content, for the four bioreactor cultures. For the FP1-fed culture (Figure 5A), the time

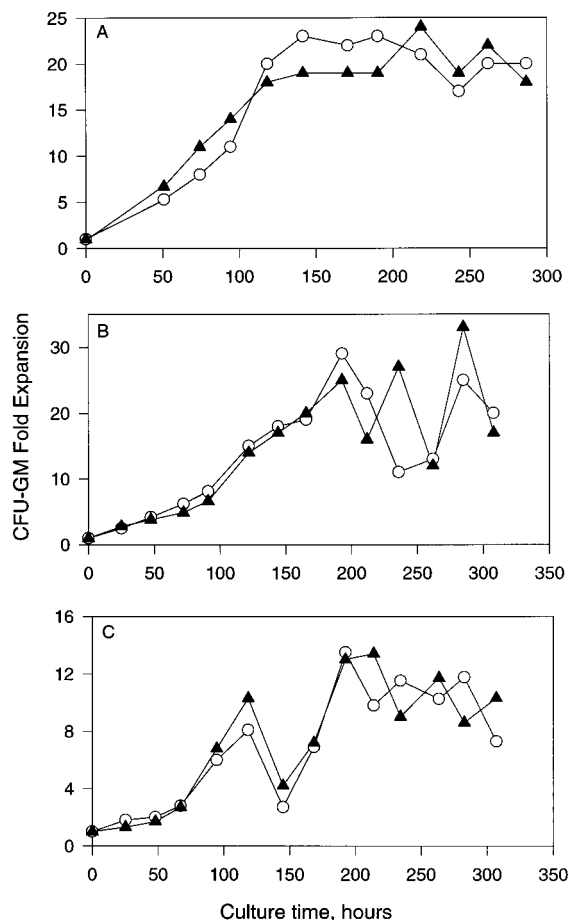


Figure 4. Time profiles for CFU-GM expansion in a T-flask (▲) and a bioreactor (○) with FP2 for (A) experiment 2, (B) experiment 3, and (C) experiment 4.

of maximum q_{lac} corresponds closely with the time of maximum % CFC and the decreasing CFC content is paralleled by decreasing q_{lac} values. These observations are consistent with those from previous experiments that utilized FP1 (Collins et al., 1997). In the current work, we observed a similar trend for q_{O_2} , although the time of maximum q_{O_2} was delayed slightly beyond that for q_{lac} . However, the times of maximum q_{lac} and % CFC did not always correspond for the FP2 cultures. The first FP2-fed CB MNC culture (Figure 5B) did exhibit an initial correspondence (through 120 h) between CFC content and q_{lac} , although q_{lac} did not decrease to a low level and a significant secondary rise in q_{lac} was observed. The sole PB MNC culture (Figure 5D) exhibited good correspondence between q_{lac} and % CFC. In contrast, the q_{lac} trend observed for the CB MNC culture shown in Figure 5C is quite different. Although an increase in q_{lac} after 150 h is noted in Figure 5, parts A, B, and possibly D, it follows an earlier peak in q_{lac} . In Figure 5C q_{lac} increases steadily from the beginning of the culture and is still increasing at the end of the culture period. Interestingly, q_{O_2} shows a much smaller (if any) secondary increase after the initial decline. As a result, the q_{O_2} profile more closely corresponds to the % CFC profile than does the q_{lac} profile for the CB MNC FP2 cultures.

The q_{lac} and q_{O_2} profiles in the bioreactor suggest two things: (1) that cell types other than CFC may impact metabolic rates, particularly q_{lac} , and (2) that the extent to which different metabolic pathways are utilized for energy generation changes with cell differentiation. We therefore examined the ratio of lactate production to oxygen consumption ($Y_{lac,ox}$). Higher $Y_{lac,ox}$ values indicate

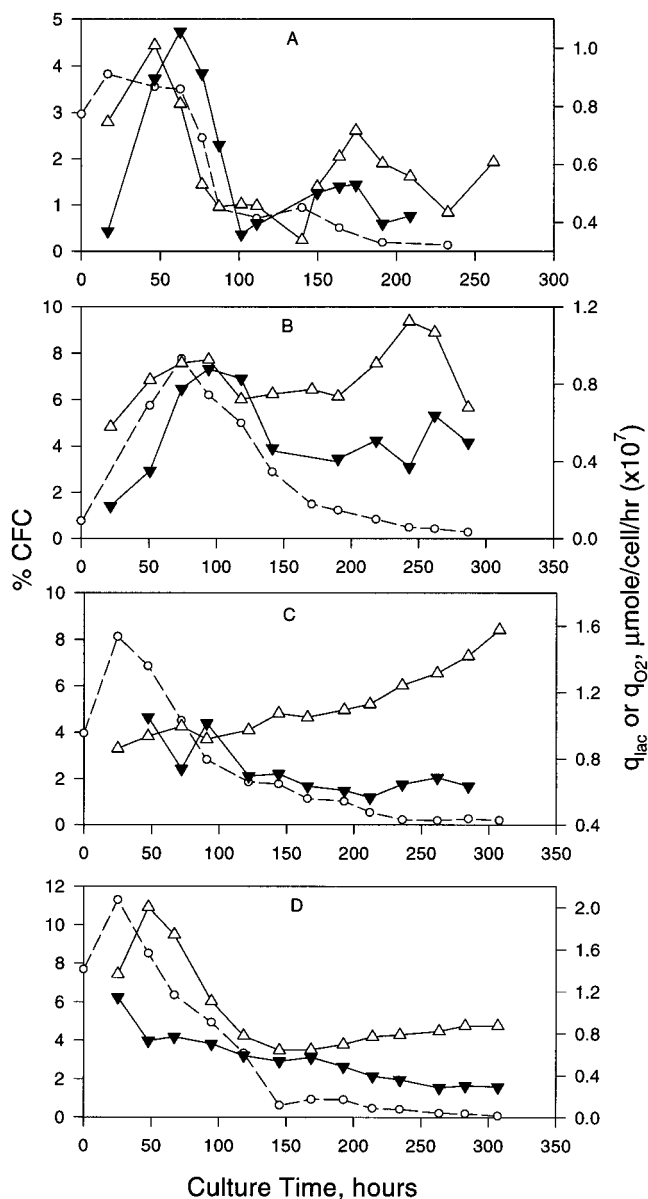


Figure 5. Time profiles for % CFC (○), q_{lac} (Δ), and q_{O_2} (▼) for (A) experiment 1, (B) experiment 2, (C) experiment 3, and (D) experiment 4.

a shift to glycolytic energy production, while lower $Y_{lac,ox}$ values suggest increased utilization of oxidative phosphorylation. Figure 6 shows the profiles for $Y_{lac,ox}$, along with the % CFC for each culture. In general, $Y_{lac,ox}$ dropped from a high value early in culture and reached a minimum when the % CFC began to plateau at a low value. Thereafter, $Y_{lac,ox}$ increased again, which suggests that post-progenitor cells rely to a greater extent on glycolysis for their energy needs. Indeed, in the one experiment analyzed using flow cytometry (Figure 6C), we observed that the increase in $Y_{lac,ox}$ (and q_{lac} , Figure 5C) was paralleled by an increase in the total percentage of CD11b⁺ and/or CD15⁺ cells. Post-progenitor cells of the granulomonocytic (GM) lineage, such as developing and mature monocytes and granulocytes, are included in the CD11b⁺ and CD15⁺ populations. We also observed that the yield of lactate on glucose increased after 150 h in culture (data not shown), further supporting the idea that post-CFCs extensively utilize glycolysis for energy production.

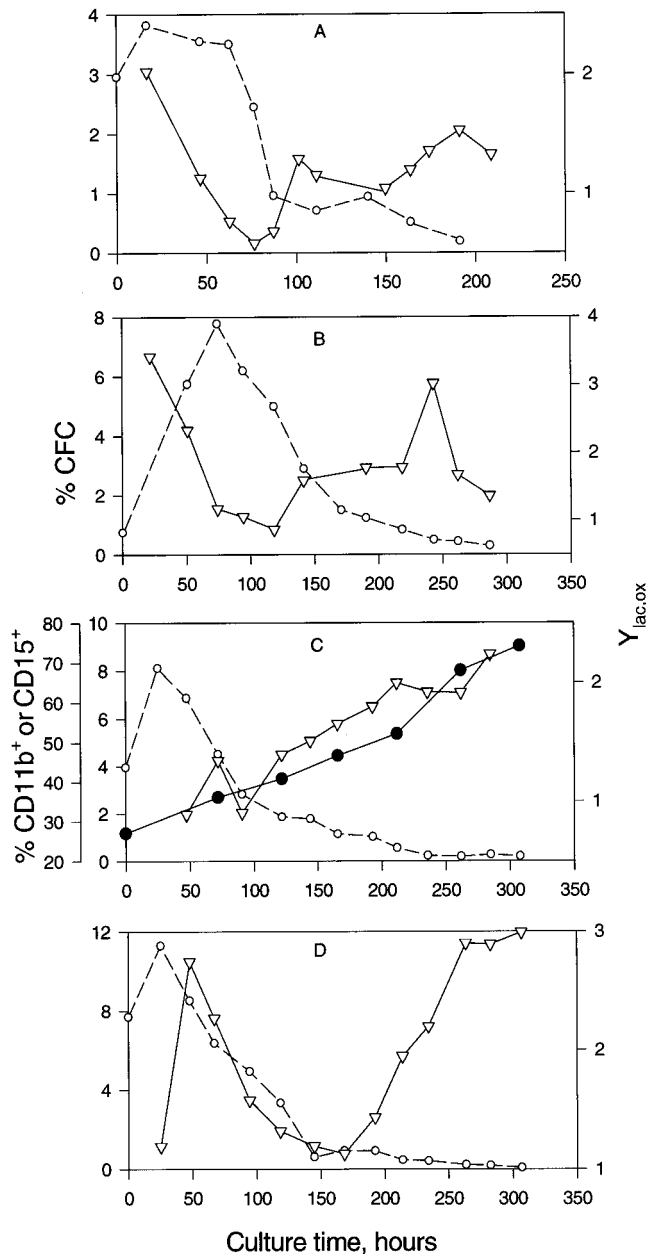


Figure 6. Time profiles for % CFC (○), $Y_{lac,ox}$ (▽), and % CD11b⁺ and/or CD15⁺ cells (●) for (A) experiment 1, (B) experiment 2, (C) experiment 3, and (D) experiment 4.

Discussion

We previously demonstrated that both CB and PB MNCs can be cultured in spinner flasks under a variety of conditions (Collins et al., 1998). Here, we demonstrate that a bioreactor vessel can be adapted to provide an acceptable culture environment for hematopoietic cells. To our knowledge, this is the first report of a stirred bioreactor with pH and DO control being used for the expansion of myeloid-lineage hematopoietic cells. This work therefore represents the next step in the evolution of hematopoietic culture from static systems to spinner culture to a fully instrumented, stirred bioreactor. Total cell expansion in the bioreactor was similar to that for past experiments in spinner flasks using the cell-retention (FP1) feeding protocol. However, CFU-GM expansion for the sample used in experiment 1 was less than the ~15-fold expansion typically observed in spinner flasks for CB MNC using FP1 (not shown).

Frequent, dilution-type feeding (FP2) increased the expansion of total cells and CFU-GM, relative to those

Table 1. Maximum Observed Total Cell Expansion, CFU-GM Expansion, and % CFU-GM for FP1 and FP2 Cultures

	maximum total cell expansion	maximum CFU-GM expansion	maximum % CFU-GM
CB MNC			
experiment 1 (FP1), bioreactor/T-flask	9.9/6.4	1.6/4.7	1.1/1.4
experiment 2 (FP2) bioreactor/T-flask	34.2/26	23.5/23.7	4.71/4.6
experiment 3 (FP2) bioreactor/T-flask	201/207	29/33	3.2/3.05
PB MNC			
historical control, ^a spinners (FP1)	14.4 ± 6.1	6.8 ± 3.2	1.61 ± 0.33
experiment 4 bioreactor (FP2)	397	13.5	5.6

^a The historical control value is the average ± 1 SEM for five ($n = 5$) PB MNC spinner flask cultures carried out in HLTM + IL-3, IL-6, SCF, G-CSF, GM-CSF, and Epo at an ID of $(2.15 \pm 0.09) \times 10^5$ cells/mL. The historical controls were fed using FP1 and represent the typical expansion we have seen in this type of system.

using the FP1 feeding protocol, for CB MNC cultures (Table 1 and data not shown for FP1 spinner flasks). Similarly, the maximum expansions of total cells and CFU-GM for the one PB MNC FP2 bioreactor culture (experiment 4) were greater than those in PB MNC FP1 spinner-flask historical controls (Table 1). Dilution-type feeding protocols have been utilized for static CD34⁺ cell cultures by many researchers (Moore and Hoskins, 1994; Lill et al., 1994; Haylock et al., 1992), with total cell expansion ratios that often exceed 1000. In static cultures of MNC and CD34⁺ cells, expansion ratios and the kinetics of expansion are highly dependent on the cell inoculum density (ID) (Koller et al., 1996; Haylock et al., 1995). In general, lower ID leads to greater total cell expansion and greater depletion of CFC. Higher ID cultures achieve greater total cell and CFC numbers, but a lower total cell expansion ratio, when compared to lower ID cultures. Perhaps due to the high residual cell density ($\geq (1.5-2) \times 10^6$ cells/mL) in our FP2 cultures, increased total cell expansion did not come at the expense of CFU-GM depletion. As shown in Table 1, the maximum observed percentages of CFU-GM (cloning efficiency) in the FP2 cultures were much greater than those observed for the FP1 bioreactor culture (CB MNC) or spinner-flask historical controls (PB MNC).

Increased total cell production with no decrease (or even an increase) in CFC production suggests that feeding by dilution released the cells from a "blocked" state—perhaps induced by accumulation of endogenous cytokines or inhibitory metabolites in FP1 cultures, which reach $(6-12) \times 10^6$ cells/mL. Although the underlying mechanisms are not well understood, decreased metabolic activity in high-density cultures has previously been reported for cells of hematopoietic origin and has been termed a "crowding" effect (Sand et al., 1977). Additional evidence for the concept of release from inhibition is provided by differences in the lactate production pattern for FP2 cultures, with a secondary increase in q_{lac} after the decline in % CFC (see below).

For FP2, total cell (Figure 3) and CFU-GM (Figure 4) expansion were similar in the bioreactor and T-flask cultures. In contrast, using FP1, we previously observed superior total cell and CFU-GM expansion in stirred (spinner) vessels vs T-flasks for PB MNC cultures inoculated with 1.2×10^6 cells/mL (Collins et al., 1998).

BM MNC cultures in perfused T-flasks benefit (in terms of total cell and CFU-GM expansion) from a similar dilution-feeding protocol (Oh et al., 1994). In that perfused T-flask system, repeated cell removal increased the available culture surface area and alleviated mass transfer limitations. Similarly, the enhanced mass transfer in stirred culture will minimize gradients in DO and pH (via increased CO₂ removal), as well as inhibitory cytokines that may accumulate in the cultures. The beneficial effect of increased mass transfer is less important at lower cell densities, as evidenced by similar total cell and CFU-GM expansion in spinner flasks vs T-flasks using FP1 in PB MNC cultures inoculated with 2×10^5 cells/mL (Collins et al., 1998). Thus, by frequently diluting the cell density in the FP2 experiments reported here, we offset the benefit of stirred culture on cell and CFU-GM production. This is consistent with the observation that the benefit of increasing medium exchange rate in static BM MNC cultures is greater at higher ID (Koller et al., 1996). Together, these observations suggest that a controlled cell density is indeed beneficial for the proliferation of hematopoietic cultures.

We previously demonstrated that CFCs consume more glucose and generate more lactate on a per cell basis than do more mature cells (Collins et al., 1997). Here we show that oxygen consumption is also higher for CFCs (Figure 5). The q_{O_2} values for our stirred cultures ranged from 1.7×10^{-8} to 1.2×10^{-7} $\mu\text{mol}/(\text{cell}\cdot\text{h})$. The highest values are slightly lower than the $(1-3) \times 10^{-7}$ $\mu\text{mol}/(\text{cell}\cdot\text{h})$ reported for hybridomas (Wohlpert et al., 1991; McQueen and Bailey, 1990; Glacken et al., 1988; Miller et al., 1987) and are much higher than the $(1-4) \times 10^{-8}$ $\mu\text{mol}/(\text{cell}\cdot\text{h})$ reported for murine bone marrow cells (Lutton et al., 1972; Olander, 1972; Gesinski and Morrison, 1968; Gesinski et al., 1968), human bone marrow cells (Peng and Palsson, 1996), and normal human granulocytes (Bird et al., 1951). The values reported for murine cells were obtained in experiments without the growth factor stimulation present in our cultures. Since growth factor stimulation increases glucose uptake in culture (Whetton et al., 1984; 1986; Spielholz et al., 1995; Hamilton et al., 1986), it is likely that cytokine stimulation would also increase q_{O_2} . The values reported for human BM MNC culture, which are much lower than those reported here, were obtained in growth-factor-supplemented systems (Peng and Palsson, 1996). However, some of the q_{O_2} values may have been obtained under oxygen-limited conditions, which would decrease the value of q_{O_2} (Miller et al., 1987; Sand et al., 1977). Interestingly, the q_{O_2} value for normal human granulocytes (2.2×10^{-8} $\mu\text{mol}/(\text{cell}\cdot\text{h})$; Bird et al., 1951) is similar to the q_{O_2} values that we observe when CFCs have been depleted.

For FP1 cultures, we almost always observe a correlation between q_{lac} and % CFC—with a close correspondence between the maxima in q_{lac} and % CFC and a low value for q_{lac} after the CFCs are depleted (Collins et al., 1997). For most FP2 cultures, there was still a maximum in q_{lac} near the maximum in % CFC, but in both FP2 CB MNC cultures, there was a secondary increase in q_{lac} that exceeded the initial peak value. This suggests that GM post-progenitors can also exhibit significant glycolytic activity and that the correlation between q_{lac} and % CFC developed for FP1 cultures (Collins et al., 1997) is not fully valid for FP2 cultures. However, this is not as great a limitation as it may seem because q_{O_2} does not exhibit a significant secondary peak after the peak in % CFC (Figure 5). This suggests that GM post-progenitors are highly glycolytic but do not have high oxidative metabolic activity. In this regard, the percentage of cells that

stained positive for CD15 and/or CD11b (GM markers) increased proportionally with $Y_{lac,ox}$ in one CB MNC FP2 culture analyzed via flow cytometry (Figure 6C). Although we did not perform flow cytometry analysis for the other bioreactor experiments, we observed similar increases in $Y_{lac,ox}$. Both monocytes and granulocytes developed concurrently in experiment 3; therefore, determining which cell type had the largest effect on $Y_{lac,ox}$ and q_{lac} is difficult. It is possible that cell types not monitored in the current research may also affect these parameters. In any event, the above results suggest that by using a variety of metabolic indicators it will be possible to estimate the content of several different cell types.

The control of pH, DO, and other physicochemical parameters in a stirred bioreactor will allow for more refined studies regarding the effects of these parameters on hematopoietic cells. Production of the large numbers of hematopoietic cells desired for clinical applications may well benefit from feeding protocols that help control the concentration of all species in a culture. Stirred bioreactor systems are readily adaptable to perfusion feeding protocols, which allow for extensive medium replacement while maintaining a high cell density. Our results suggest that monitoring metabolic quotients for oxygen, lactate, and other metabolites will allow practitioners to estimate the percentages of both CFCs and more mature cells in culture. The ability to characterize on-line which hematopoietic populations are present at any particular time would allow clinicians to harvest cultures when the desired cell type(s) is at a maximum, thus benefiting patient recovery.

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