Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12140–12144, December 1994
Cell Biology

Establishment of an adherent cell feeder layer from human umbilical cord blood for support of long-term hematopoietic progenitor cell growth

(hematopoiesis/stromal cell layer/growth factor/stem cell)

Z.-Q. YE*, J. K. BURKHOLDER†, P. QIU†, J. C. SCHULTZ*, N. T. SHAHIDI*‡, AND N.-S. YANG†

*Hematology/Oncology Division, Department of Pediatrics, University of Wisconsin, Madison, WI 53792; and †Cancer Gene Therapy, Agracetus, Inc., 8520 University Green, Middleton, WI 53562

Communicated by C. C. Tan, July 11, 1994

ABSTRACT **Previous attempts to establish a stromal cell feeder layer from human umbilical cord blood (HUCB) have met with very limited success. It has been suggested that there is an insufficient number of stromal precursor cells in HUCB to form a hematopoietic-supporting feeder layer in primary cultures. The present study shows that HUCB does contain a significant accessory cell population that routinely develops into a confluent, adherent cell layer under defined primary culture conditions. HUCB-derived adherent layers were shown to support long-term hematopoietic activity for an average of 4 months. This was achieved by using a customized coverslip with a modified surface structure as the cell attachment substratum and using a specialized culture feeding regime. We have characterized the various cell types (including fibroblasts, macrophages, and endothelial cells) and extracellular matrix proteins (including fibronectin, collagen III, and laminin) that were present in abundance in the HUCB-derived adherent cell layer. In contrast, oil red O-staining fat cells were rarely detected. ELISA and bioassays showed that stem cell factor and interleukin 6 were produced by the HUCB stromal cell cultures, but interleukin 3 or granulocyte/macrophage colony-stimulating factor was not detected. Application of this hematopoietic culture system to transgenic and gene therapy studies of stem cells is discussed.**

There is now ample evidence that effective hematopoiesis is the product of interaction between the hematopoietic stem cells and the supporting stroma that provide the necessary regulatory factors for the maintenance and proliferation of hematopoietic progenitors (1–3). It is well known that in long-term liquid culture of the bone marrow, the maintenance and differentiation of hematopoietic stem cells are dependent on the establishment of a foundation of adherent cells (4, 5). Recent interest in human umbilical cord blood (HUCB) as a source of hematopoietic stem cells for transplantation and gene therapy has prompted investigators to evaluate the growth potential of HUCB-derived hematopoietic stem cells in long-term culture (6, 7). Utilizing an irradiated preformed bone marrow-derived stromal layer, it has been possible to maintain active hematopoiesis in HUCB cultures up to about 16 weeks (8). However, the establishment of long-term hematopoietic cell cultures using a stromal layer from HUCB has consistently yielded poor results. Some investigators have ascribed this lack of a viable microenvironment to sustain hematopoiesis to an inadequate number of stromal precursor cells in the HUCB (8, 9).

In this study we evaluated the ability of HUCB stromal cells to form a functional feeder layer and support hematopoiesis over a long period in culture. Our findings suggest that, under certain culture conditions, a functional feeder layer can be established routinely and that such a feeder layer can support hematopoiesis, in the absence of any exogenous growth factor, for as long as 16 weeks. Characteristics of culture behavior, cell dynamics, long-term maintenance, and differentiation of hematopoietic progenitors in the HUCB-derived adherent cell layer system are described. The potential application of this technology to stem cell gene transfer and gene therapy is also discussed.

MATERIALS AND METHODS

Medium and Reagents. Complete medium for long-term culture was prepared by supplementing Iscove's modified Dulbecco's medium (IMDM) with 12.5% fetal bovine serum (FBS; Sigma), 12.5% horse serum (GIBCO), 50 μM 2-mercaptoethanol, 2 mM L-glutamine, and 1 μM hydrocortisone sodium succinate. All serum was heat-inactivated and stored frozen prior to use. In some experiments, stem cell factor (SCF; R & D Systems), interleukin 3 (IL-3; R & D Systems), and interleukin 6 (IL-6; Sigma) were added to test cell cultures at concentrations of 50 ng/ml, 2.5 ng/ml, and 5 ng/ml, respectively.

Cell Source and Cell Separation Procedures. HUCB samples were kindly provided by the Obstetrics Department of the Meriter Hospital, Madison, WI. After a signed consent form was approved by the Institutional Review Board, bone marrow samples were obtained from healthy donors by bone marrow puncture. Upon receipt of heparinized HUCB or bone marrow samples, the cells were diluted with IMDM, layered onto Lymphoprep (1.077 ± 0.001 g/ml; Nycomed, Oslo), and centrifuged at 300 × *g* for 20 min. Cells from the gradient interface were collected and washed three times in IMDM and then resuspended in medium for culture.

Initiation and Maintenance of Long-Term Cultures. Mononuclear cells were suspended in complete culture medium at a density of 2×10^6 cells per ml, and 2 ml per well was dispensed into 12-well tissue culture plates (Corning). An autoclaved, custom-made 19-mm (diameter) coverslip was placed at the bottom of each 25-mm culture well to serve as the substratum for growth of the adherent cell layer. These specialized, custom-made glass coverslips are commercially available from Midway Culture-Tech (Mundelein, IL; catalog no. MCT001). According to Jonathan Chen, who developed this specialty coverslip, a higher temperature than normally used for the production of standard coverslips is used in the manufacturing procedure. For growth and maintenance of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HUCB, human umbilical cord blood; SCF, stem cell factor; IL-3, interleukin 3; IL-6, interleukin 6; GM-CSF, granulocyte/macrophage colony-stimulating factor; CFU-GM, granulocyte/macrophage colony-forming unit(s); CFU-E, erythroid CFU(s); BFU-E, erythroid burst-forming unit(s).

‡To whom reprint requests should be addressed.

testing cell samples, all cultures were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 in air. Weekly feeding was performed by replacing half of the culture medium with fresh culture medium. The nonadherent cells from the culture were harvested for morphological studies and colony-forming unit assays at the indicated times.

Methylcellulose Colony-Forming Unit Assays. Cells from weekly culture harvests were washed with phosphate-buffered saline (PBS), counted, and suspended in IMDM. Cells were plated at a density of 1×10^5 per ml in Iscove's methylcellulose plating mixture (HCC-4433; Stem Cell Technologies, Vancouver), which contains a complete pretested mixture of methylcellulose, FBS, bovine serum albumin, erythropoietin, and phytohemagglutinin-stimulated human leucocyte conditioned medium. Aliquots of 0.5 ml were plated in duplicate or triplicate into 24-well plastic culture plates and incubated under fully humidified conditions in a 5% CO_2 atmosphere at 37°C. After 21 days, granulocyte/macrophage colony-forming units (CFU-GM), erythroid CFUs (CFU-E), erythroid burst-forming units (BFU-E), and CFU-Mix were scored as described (8).

Immunofluorescence Staining. The adherent cell layers grown on customized coverslips were washed with PBS and then incubated with monoclonal antibodies for 45 min at room temperature as described (10). Primary antibodies used were mouse anti-human $CD14^+$ (Becton Dickinson), rabbit anti-human fibronectin (Cappel), goat anti-collagen III (Fisher), and rat anti-laminin (Immunotech, Westbrook, ME). A mouse anti-human growth hormone monoclonal antibody was used as a negative control. Samples were washed and stained with the appropriate secondary antibodies, including sheep anti-mouse IgG-phycoerythrin (PE) (Molecular Probes), goat anti-rat IgG-fluorescein isothiocyanate (FITC) (Immunotech), rabbit anti-goat IgG-PE (Fisher), and sheep anti-rabbit IgG-FITC (Cappel). Titers of primary and secondary antibodies for each specific staining are described in the text and as referenced (10). The samples were then analyzed by immunofluorescence microscopy.

Proescher's Oil Red Staining. The adherent cell layer on the coverslip was fixed with formalin, transferred to pyridine for 3–5 min, and then stained in oil red O solution for 30 min as described (11). The samples were observed by light microscopy after counterstaining with hematoxylin.

Scanning Electron Microscopy. Adherent cell layers grown on coverslips were washed with PBS, fixed with 1% glutaraldehyde and 0.5% tannic acid, then stained with 1% OsO_4. Samples were dehydrated through an ethanol series, critical point dried, coated with gold, and analyzed by scanning electron microscopy (ADEM, NORAN Instruments, Middleton, WI).

Table 1. Effect of culture substratum on establishment of adherent cell layer from HUCB

Source of adherent layer	Relative cell growth			
	Poly(D-lysine)-coated plastic*	Standard plastic surface†	Regular coverslip‡	Special coverslip§
HUCB	− to 2+	− to 2+	− to 2+	4+
Bone marrow	NT	4+	NT	4+

Aliquots of a 2-ml mononuclear cell suspension (2×10^6 cells per ml) were plated and grown as described in the text. At 5 weeks after plating, adherent cells attached to culture substrata were stained with crystal violet and relative cell growth was evaluated semiquantitatively. 1+, 2+, 3+, and 4+ denote a 10–25%, ≈50%, ≈75%, and ≥80% confluency of stromal cells in culture grown on 19-mm coverslips, respectively. NT, not tested.

*Poly(D-lysine)-coated plastic: tissue culture treated plastic coated with poly(D-lysine) [Collaborative Biomedical Products (Bedford, MA); M_r 500,000–550,000].

†Standard plastic surface: tissue culture treated plastic, Corning no. 25815.

‡Regular coverslip: Baxter Scientific Products (McGaw Park, IL), no. M6047-2.

§Special coverslip: Midway Culture-Tech customized coverslip.

Quantitative Assessment of Growth Factors and Lymphokines in Medium Conditioned by HUCB-Derived Adherent Cell Layer Cultures. The presence of SCF, IL-3, IL-6, and granulocyte/macrophage colony-stimulating factor (GM-CSF) in the conditioned medium of HUCB-derived adherent cell layer cultures was quantitatively determined. Enzyme-linked immunosorbent assays (ELISAs) for human SCF (R & D Systems), GM-CSF (Biosource, Camarillo, CA), and IL-3 (R & D Systems) were performed according to manufacturer's instructions. Sensitivities of these assays were 4 pg/ml for SCF, 20 pg/ml for GM-CSF, and 7.8 pg/ml for IL-3. IL-6 was measured using the B_9 cell line bioassay method as reported (12). Fresh complete culture medium was used as a negative control in all assays.

RESULTS

Establishment of Adherent Cell Layers from HUCB Cultures. HUCB-derived adherent cell layers from 15 cord blood samples were successfully grown on customized coverslips. The capacity of four different culture substrata to support growth of HUCB-derived adherent layer cells was evaluated using a semiquantitative analysis of the cell confluence in

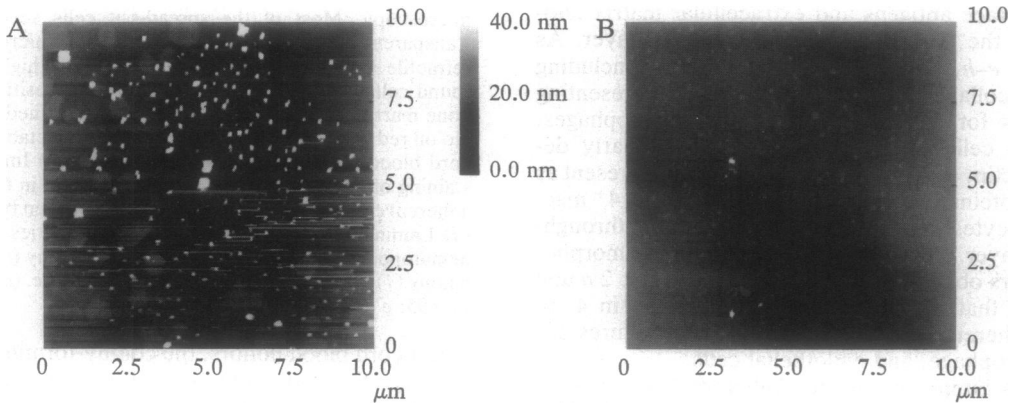

FIG. 1. Distinguishable surface properties observed between a standard glass coverslip (Baxter Scientific Products) (*A*) and the Midway Culture-Tech (MCT) custom-made glass coverslip (*B*) when examined by atomic force microscopy. Prominent granular particles and coarse bump structures were observed on the standard coverslips (*A*) but were minimally or not detectable on the Midway Culture-Tech coverslips.

culture. Table 1 shows that HUCB-derived adherent layer cells prefer the customized coverslip to standard tissue culture-treated plastic surface, the poly(D-lysine)-coated plastic surface, or the regular glass coverslip as a growth substratum. The surface structure of the customized glass coverslip was hence evaluated and compared with a regular glass coverslip using atomic force microscopy. Fig. 1 shows that the customized coverslip has few or no outstanding structures on its surface, while the regular coverslip surface has prominent granular particles or spikes and some coarse bump structures. It remains to be determined if these or other features are responsible for the very different cell attachment and growth rate observed between the customized and regular coverslips.

The HUCB-derived adherent cell layer grown on the customized coverglass reached confluence at 4–5 weeks; in comparison, the bone marrow-derived stromal cell layers took 2 weeks to reach confluence under the same culture conditions (data not shown). Exogenously added growth factors and lymphokines were tested for a stimulatory effect on the formation of HUCB-derived adherent cell layer. Weekly addition of SCF, IL-3, and IL-6, alone or in combination, did not accelerate the adherent layer formation in HUCB cultures (data not shown). A lack of a stimulatory effect on stromal layer formation was similarly observed in the bone marrow culture system.

Morphological and Cell Surface Characteristics of the HUCB-Derived Adherent Layer Cells. Cell morphological and culture behavioral properties of established HUCB long-term cultures were examined and characterized by light and scanning electron microscopy. Heterogeneous cell types apparently consisting of fibroblasts, macrophages, and endothelial cells (see below) were observed to attach firmly to the coverslip substratum as large (≥30 μm), spread-out cells (Fig. 2*a*). Above these cells, small (≤10 μm), round-shaped cells were routinely seen associated with the adherent feeder layer after it was established in culture. Under phase microscopy, larger cells (macrophages) were readily observed, and small refractile cells were also detected in large numbers in cultures containing established adherent cells (Fig. 2*b*). Our observation of small refractile cells associated with the spread-out feeder layer cells is very similar to the hematopoietic cells described in the bone marrow-derived stromal cell culture system (2). Wright's staining of such cultures revealed cells of different morphology, including undifferentiated, myeloid lineage and erythroid lineage cells. Active mitotic figures were observable in these cultures. In contrast to bone marrow stromal cell cultures, minimal or no oil red O staining positive cells were detected in the cord blood-derived cultures (Fig. 2 *c* and *d*).

Indirect immunofluorescence assays were used to analyze specific cell surface antigens and extracellular matrix antigens present in the HUCB-derived adherent cell layer. As seen in Fig. 2 *e–h*, four cell surface proteins, including fibronectin and collagen III, CD14, and laminin representing marker proteins for fibroblasts, monocytes/macrophages, and endothelial cells (13), respectively, were clearly detected. Fibronectin, collagen III, and laminin were present as extracellular proteins in a matrix network. The $CD14^+$ macrophages/monocytes were present as cells scattered throughout the feeder layer. These results concur with the morphological characters observed for these cell types (Fig. 2 *a* and *b*) and confirm that the major cell types present in 4- to 13-week-old adherent cell layers from HUCB cultures are fibroblast, macrophage, and endothelial cells.

Hematopoiesis Supported by the Adherent Cell Layer in HUCB Cultures. Hematopoietic activity was ascertained by the ability of certain nonadherent cells from HUCB cultures to form colonies after they became dissociated from the adherent layer. In seven independent experiments from different cord blood donors, the colony-forming unit-producing activity of nonadherent cells present in these cultures was monitored on a weekly basis. In this time course study (Fig. 3), test cultures were plated and grown under the standard experimental conditions (i.e., allowing establishment of adherent feeder cells but no addition of exogenous growth

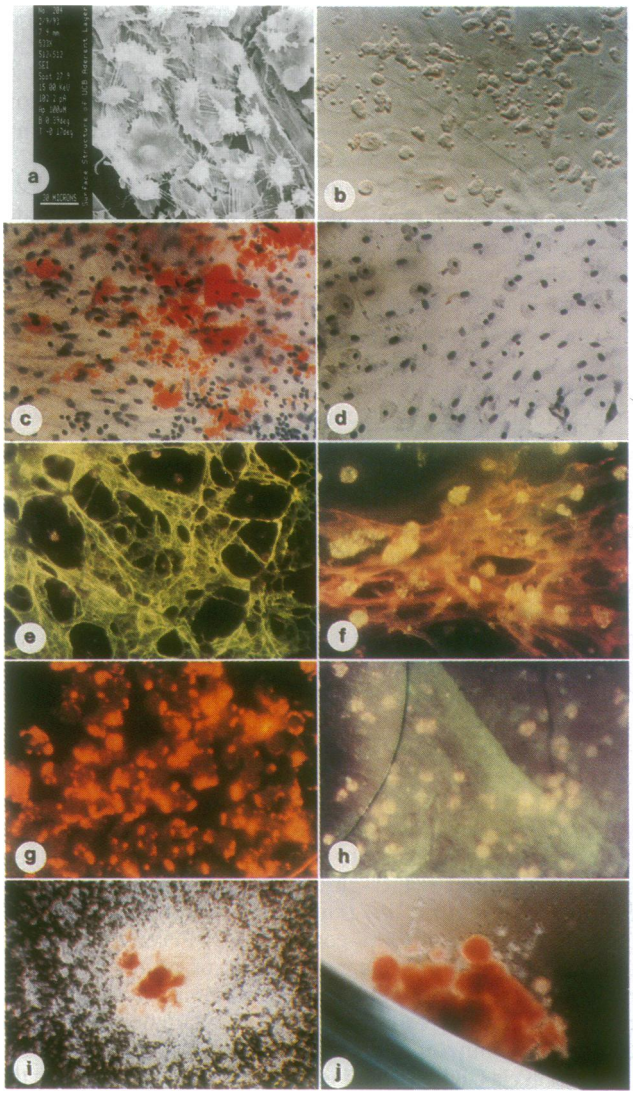

FIG. 2. Morphological, cell attachment, culture behavior, and immunochemical characteristics of cord blood-derived adherent layer cells examined under microscopy. (*a*) Scanning electron microscope photomicrograph shows various cell types are present as an adherent cell layer in this culture system (7 weeks in culture). The fibroblasts, endothelial cells, and macrophages (see *c–f* for confirmation of cell types) were present as firmly attached monolayer cells with a diameter of ≥30 μm, while the much smaller (≤10 μm) round cells attached on top of the spread-out "feeder layer" cells apparently are the hematopoietic progenitor (HP) cells. (*b*) Morphological appearance of the HUCB-derived adherent cell layer under phase microscopy. Most of the spread-out cells seen in *a* are highly transparent when observed under phase microscopy: the more refractile large-size cells are macrophages; the highly refractile, small round cells are the HP cells. (*c*) Oil red O-positive adipocytes in a bone marrow stromal layer (7 weeks old) stained bright orange. (*d*) No oil red O-positive-staining cells were detectable in a 10-week-old cord blood-derived adherent cell layer. (*e–h*) Immunofluorescence staining of cell type-specific antigens present in the HUCB-derived adherent cell layer. (*e*) Fibronectin. (*f*) Collagen type III. (*g*) $CD14^+$. (*h*) Laminin. (*i* and *j*) Colony-forming activities of HUCB-derived hematopoietic cultures: a CFU–Mix cell colony (*i*) and a BFU-E cell colony (*j*) grown out in methylcellulose culture. (*a*, ×320; *b–d*, *f*, and *g*, ×95; *e* and *h*, ×60; and *i* and *j*, ×20.)

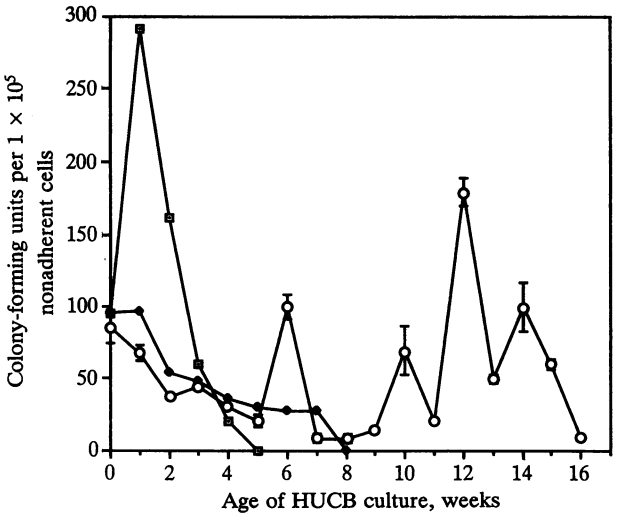

FIG. 3. Hematopoietic activity in long-term cord blood cultures supported by the HUCB adherent cell layer. The total number of colony-forming units from 1 × 10^5 nonadherent cells plated in methylcellulose culture, including CFU-Mix, BFU-E, CFU-E, and CFU-GM, are presented. ○, HUCB adherent layer, without exogenous growth factors; ⊡, no adherent layer, with exogenous growth factors; ◆, no adherent layer, without exogenous growth factors. Clonogenic assay results from one donor are shown; similar patterns were obtained from six independent donors. Each time point represents data from triplicate methylcellulose cultures ± standard deviation. To avoid complexity, standard deviations of the control sets are not shown.

factors). Cultures maintained without the adherent layer and with or without weekly additions of SCF, IL-3, and IL-6 were used as parallel controls in each experiment. We observed that in cultures containing an established adherent layer, production of colony-forming unit was sustained for an average of 16 weeks (Fig. 3). During this long-term culture period, substantial hematopoietic activity was observed. Furthermore, when the adherent cell layer was removed from test cultures at the fifth or eighth week in culture, colony-forming progenitor cells quickly diminished within 2–3 weeks (data not shown). As shown in Fig. 2 *i* and *j*, at 12 weeks in culture the distribution of different cell populations capable of colony-forming activity was quantitatively analyzed, and the ratio of CFU-Mix/BFU-E/CFU-E/CFU-GM was determined to be 13:23:17:47 (average of five independent experiments). Together these results demonstrate the presence of active hematopoiesis in these long-term cultures. In cultures that did not contain an adherent cell layer, but were supplemented weekly with exogenous growth factors, the colony-forming activity reached a high level during the first 2 weeks but declined rapidly in the following 2 weeks and disappeared by the fifth week. In cultures without added growth factors and without an adherent cell layer the yield of colony-forming unit was low, and hematopoietic activity disappeared by 8 weeks following culture initiation. The results shown in Fig. 3 therefore demonstrate that healthy, actively proliferating hematopoietic progenitor cells are present in this HUCB-derived culture system and that proliferation and maintenance of such hematopoietic activity in long-term cultures depends specifically on the establishment of an adherent feeder cell layer.

Presence of Endogenous SCF and IL-6 in Cord Blood Cultures Containing an Adherent Cell Layer. Prior to the regular weekly feeding, conditioned medium samples from long-term cord blood cultures were collected at 6, 9, and 11 weeks after culture initiation. Results shown in Table 2 demonstrate that endogenous SCF and IL-6 were readily detectable in our standard HUCB cultures. However, the presence of IL-3 and GM-CSF was not detected using the currently employed ELISA systems.

Table 2. Quantitative assessment of endogenous growth factors produced by a HUCB-derived adherent layer culture system

Time in culture, weeks	Endogenous growth factor, pg/ml			
	SCF	IL-6	IL-3	GM-CSF
6	35	103	ND	ND
9	20	NT	ND	ND
10	31	70	NT	NT
11	28	68	NT	NT

Replicate HUCB cell cultures containing an initial cell number of 4 × 10^6 cells in 2 ml were plated, grown out, and maintained as described in the legend to Table 1. The presence of growth factors in conditioned media collected from test cultures was analyzed by ELISA or bioassay as described in the text. ND, not detectable; NT, not tested.

DISCUSSION

Comparative studies of hematopoietic progenitor cells in cord blood and bone marrow samples have shown a higher $CD34^+$, $CD38^-$ cell fraction in HUCB than in normal adult bone marrow, suggesting that the very primitive progenitor cells may be more abundant in cord blood (14). Indeed, HUCB has been successfully used as an effective alternative to bone marrow for hematopoietic reconstitution (15). As a result, there has been a great deal of interest in the growth potential and expansion of HUCB *in vitro*. However, previous attempts to establish long-term hematopoietic cell cultures using a stromal layer from HUCB have proven unsuccessful. In fact, some researchers have previously indicated that cord blood may not contain a sufficient stromal cell population (8, 9). Using the specific culture conditions defined in this study, we have demonstrated here that HUCB cells can now be routinely cultured to form a confluent adherent feeder layer. This layer shares many characteristics with bone marrow-derived stromal layer, including the various constituent cell types, culture behaviors, the presence of extracellular matrix proteins (Fig. 2 *e–h*), and the ability of these cells to support long-term *in vitro* hematopoiesis (Fig. 2 *i* and *j*; Fig. 3).

The present HUCB cell culture system requires a special culture substratum for the attachment and formation of an adherent cell layer. The differences in surface structure observed between the regular and the customized glass coverslips are significant, but it is not clear how these differences contribute to the difference in attachment and growth rates of the adherent layer cells in HUCB cultures. After the adherent cell layer is established, a large number of round and refractile hematopoietic cells were found to attach on top of these feeder layer cells. Scanning electron microscopy (Fig. 2*a*) revealed that a close cell-to-cell association mediated by "prickle or spike" structures connecting the hematopoietic cells and feeder layer cells (Fig. 2*a*) was evident in this HUCB culture system.

An interesting feature of the HUCB-derived adherent layer is the reduction or lack of a population of adipocytes when compared to bone marrow stroma. Although the physiological function of adipocytes in bone marrow stroma has not been clearly established, it has been noted that lipid-containing cells often become prominent in the later stages of long-term bone marrow cultures. Also, it was reported that an abundance of adipocytes in the early stages of long-term bone marrow culture is usually associated with poor hematopoietic activity (16). The interrelationship between the number of adipocytes and the activity of hematopoietic proliferation may be mediated by interleukin 11 (17). Our findings of a small number of adipocytes in HUCB cultures

in the presence of an exuberant hematopoietic activity are in agreement with the above observations.

It has been suggested that clonogenic cells present at the initiation stage of hematopoietic cultures must either differentiate or die (1, 3, 18). Clonogenic progenitor cells recovered after 5–8 weeks in cultures with a competent stromal feeder layer should then represent the progeny of more primitive progenitor cells, referred to as long-term culture-initiating cells (19, 20). In our experiments, HUCB cultures with a confluent adherent layer were able to maintain and amplify the outgrowth of clonogenic progenitor cells (including CFU-Mix, BFU-E, CFU-E, and CFU-GM) for an average of 16 weeks in culture. The efficiency of the HUCB-derived stromal layer, measured by the number of colony-forming units (Fig. 3), was very similar to the efficiency of the bone marrow culture system reported by Gartner and Kaplan (21). It is important to note that the hematopoietic activity in our system was detected without the addition of exogenous growth factors. In cultures that did not contain an adherent cell layer, weekly additions of exogenous growth factors (SCF, IL-3, and IL-6) resulted in a boost of colony-forming cell proliferation in the first 2 weeks. However, this activity quickly declined and subsided at 5 weeks, consistent with the observations reported by Williams (22) and Migliaccio *et al.* (23). Furthermore, in the absence of an adherent cell layer and exogenous growth factors, the cord blood cultures grew poorly, and the low levels of clonogenic progenitor cells disappeared at 8 weeks. Similar observations were reported by Hows *et al.* (8) and Strobel *et al.* (9). These results are also consistent with our observation that after removal of the preformed adherent layer from the culture, the activity of colony-forming cells disappears within 2–3 weeks. Based on the above results, we therefore conclude that the adherent cell layer plays an important role in supporting the growth, maintenance, differentiation, and possibly the renewal of hematopoietic progenitor cells in HUCB-derived cultures.

Previous studies have shown that SCF, IL-3, and IL-6 play a critical role in the amplification and differentiation of progenitor hematopoietic cells (22–25). We show here that significant levels of endogenous SCF and IL-6 are detectable in the HUCB-derived adherent layer cultures. Using sensitive ELISAs, we failed to detect endogenous GM-CSF and IL-3 in long-term HUCB cultures. It has been reported that adherent cell lines derived from mouse bone marrow are able to stimulate multipotential progenitor cells in the absence of exogenous and detectable endogenous IL-3 (26). It was also reported that GM-CSF was not detected in conditioned medium from primary human bone marrow stromal cultures (27). These findings and our present results indicate that IL-3 and GM-CSF may not be essential in stimulating the hematopoietic potential of the HUCB-derived cultures containing the adherent feeder layer. Our results therefore strongly suggest that the HUCB-derived adherent layer plays a *bona fide* supportive role for long-term hematopoiesis in culture, which cannot be effectively replaced by weekly addition of exogenous SCF, IL-3, and IL-6.

In this study, we demonstrate that there is a significant hematopoietic accessory cell population in HUCB samples that can, under the appropriate culture conditions, form a functional feeder layer to support long-term hematopoiesis. The HUCB-derived adherent cell layer culture system developed in this study provides an alternative approach for establishment and manipulation of long-term HUCB progenitor cell cultures. This may allow investigators to more effectively study the mechanisms of cell development, maintenance, and differentiation of fetal and neonatal hematopoietic stem cells. Our recent studies utilizing gene gun technology have shown that $CD34^+$ and other cell types present in HUCB samples can be effectively transfected with various reporter and lymphokine genes (28). The *in vitro* expansion of transfected $CD34^+$ cells on an autologous feeder layer would be far superior to a bone marrow-derived adherent layer, for it would avoid the possibility of allogeneic cell contamination.

1. Sutherland, H. J., Lansdorp, P. M., Henkelman, D. H., Eaves, A. C. & Eaves, C. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3584–3588.
2. Golde, D. W. (1991) *Sci. Am.* **265**, 86–93.
3. Verfaillie, C., Blakolmer, K. & McGlave, P. (1990) *J. Exp. Med.* **172**, 509–520.
4. Dexter, T. M. (1982) *J. Cell. Physiol. Suppl.* **1**, 87–94.
5. Rowley, S. D., Brashem-Stein, C., Andrews, R. & Bernstein, I. D. (1993) *Blood* **82**, 60–65.
6. Broxmeyer, H. E., Douglas, G. W., Hangoc, G., Cooper, S., Bard, J., English, D., Arny, M., Thomas, L. & Boyse, E. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3828–3832.
7. Moritz, T., Keller, D. C. & Williams, D. A. (1993) *J. Exp. Med.* **178**, 529–536.
8. Hows, J. M., Bradley, B. A., Marsh, J. C. W., Luft, T., Coutinho, L., Testa, N. G. & Dexter, T. M. (1992) *Lancet* **340**, 73–76.
9. Strobel, E.-S., Gay, R. E. & Greenberg, P. L. (1986) *Int. J. Cell Cloning* **4**, 341–356.
10. Rudnicki, M. A. & McBurney, M. W. (1987) in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, ed. Robertson, E. J. (IRL, Oxford), p. 45.
11. Clark, G. (1981) in *Staining Procedures*, ed. Clark, G. (Williams & Wilkins, Baltimore), p. 193.
12. Ershler, W. B., Sun, W. H., Binkley, N., Gravenstein, S., Volk, M. J., Kamoske, G., Klopp, R. G., Roecker, E. B., Daynes, R. A. & Weindruch, R. (1993) *Lymphokine Cytokine Res.* **12**, 225–230.
13. Andreoni, C., Moreau, I. & Rigal, D. (1990) *Exp. Hematol.* **18**, 431–437.
14. Cardoso, A. A., Li, M.-L., Batard, P., Hatzfeld, A., Brown, E. L., Levesque, J.-P., Sookdeo, H., Panterne, B., Sansilvestri, P., Clark, S. C. & Hatzfeld, J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8707–8711.
15. Vilmer, E., Sterkers, G., Rahimy, C., Denamur, E., Elion, J., Broyart, A., Lescoeur, B., Tiercy, J. M., Gerota, J. & Blot, P. (1992) *Transplantation* **53**, 1155–1157.
16. Touw, I. & Lowenberg, B. (1983) *Blood* **61**, 770–774.
17. Keller, D. C., Du, X. X., Srour, E. F., Hoffman, R. & Williams, D. A. (1993) *Blood* **82**, 1428–1435.
18. Sutherland, H. J., Eaves, C. J., Eaves, A. C., Dragowska, W. & Lansdorp, P. M. (1989) *Blood* **74**, 1563–1570.
19. Issaad, C., Croisille, L., Katz, A., Vainchenker, W. & Coulombel, L. (1993) *Blood* **81**, 2916–2924.
20. Sutherland, H. J., Hogge, D. E., Cook, D. & Eaves, C. J. (1993) *Blood* **81**, 1465–1470.
21. Gartner, S. & Kaplan, H. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4756–4759.
22. Williams, D. A. (1993) *Blood* **81**, 3169–3172.
23. Migliaccio, G., Migliaccio, A. R., Druzin, M. L., Giardina, P.-J. V., Zsebo, K. M. & Adamson, J. W. (1992) *Blood* **79**, 2620–2627.
24. Koller, M. R., Bender, J. G., Papoutsakis, E. T. & Miller, W. M. (1992) *Blood* **80**, 403–411.
25. Brugger, W., Möcklin, W., Heimfeld, S., Berenson, R. J., Mertelsmann, R. & Kanz, L. (1993) *Blood* **81**, 2579–2584.
26. Li, C. L. & Johnson, G. R. (1985) *Nature (London)* **316**, 633–636.
27. Novotny, J. R., Duehrsen, U., Welch, K., Layton, J. E., Cebon, J. S. & Boyd, A. W. (1990) *Exp. Hematol.* **18**, 775–784.
28. Ye, Z.-Q., Qiu, P., Burkholder, J. K., Shahidi, N. T. & Yang, N.-S. (1993) *Blood* **82** (Suppl. 1), 217a (abstr.).