British Journal of Haematology 2000, **109**, 235–242

Mesenchymal progenitor cells in human umbilical cord blood

ALEJANDRO ERICES,[1] PAULETTE CONGET[1] AND JOSÉ J. MINGUELL[1,2] [1]*Unidad de Biología Celular, INTA, Universidad de Chile, Santiago, and* [2]*Unidad de Trasplante de Médula Osea, Clínica Las Condes, Santiago, Chile*

Received 28 September 1999; accepted for publication 11 December 1999

Summary. Haemopoiesis is sustained by two main cellular components, the haematopoietic cells (HSCs) and the mesenchymal progenitor cells (MPCs). MPCs are multipotent and are the precursors for marrow stroma, bone, cartilage, muscle and connective tissues. Although the presence of HSCs in umbilical cord blood (UCB) is well known, that of MPCs has been not fully evaluated. In this study, we examined the ability of UCB harvests to generate in culture cells with characteristics of MPCs. Results showed that UCB-derived mononuclear cells, when set in culture, gave rise to adherent cells, which exhibited either an osteoclast- or a mesenchymal-like phenotype. Cells with the osteoclast phenotype were multinucleated, expressed TRAP activity and antigens CD45 and CD51/CD61. In turn, cells with the mesenchymal phenotype displayed a fibroblast-like morphology and expressed several MPC-related antigens (SH2, SH3, SH4, ASMA, MAB 1470, CD13, CD29 and CD49e). Our results suggest that preterm, as compared with term, cord blood is richer in mesenchymal progenitors, similar to haematopoietic progenitors.

Keywords: cord blood, mesenchymal progenitors, osteoclasts, cell culture.

Haemopoiesis is sustained by two main cellular components, the haematopoietic cells (HSCs) and the mesenchymal progenitor cells (MPCs) (Tavassoli & Minguell, 1991; Orkin, 1995). MPCs, which are different from the 'typical' haematopoietic stromal cells (Chichester *et al*, 1993; Majumdar *et al*, 1998), are multipotent and serve as long-lasting precursors for bone marrow stromal cells, bone, cartilage, muscle and connective tissue (Prockop, 1997; Ferrari *et al*, 1998; Conget & Minguell, 1999; Pittenger *et al*, 1999). During development, the migratory nature of the haematopoietic process implies that both cellular components should be able to mobilize through the blood, thus allowing the transit of haemopoiesis from an immature into a more mature haemopoietic site (Tavassoli, 1991).

The circulatory capacity of the haematopoietic stem cell is well documented in adults by transplantation studies and supported by the observation that these cells circulate in umbilical cord blood (Rubinstein *et al*, 1995; Wyrsch *et al*, 1999) and, under certain circumstances, in peripheral blood (Siena *et al*, 1989). In turn, although direct evidence for the presence of MPCs in the blood does not exist, the observation that after transplantation MPCs functionally engraft in the marrow (Horwitz *et al*, 1999) has been considered as proof of their circulatory and homing capacity. In addition, mesenchymal progenitors have been shown to circulate in cytokine-mobilized blood of cancer patients (Fernández *et al*, 1997).

As there is no information on whether mesenchymal progenitor cells circulate in umbilical cord blood (UCB), in this study we evaluated the ability of human UCB harvests to give rise *in vitro* to a population of cells exhibiting the characteristics of mesenchymal progenitors.

Correspondence: Dr Alejandro Erices, Unidad de Biología Celular, INTA, Universidad de Chile, Casilla 138, Santiago 11, Chile. E-mail: aerices@uec.inta.uchile.cl

MATERIALS AND METHODS

Cord blood harvest and preparation of adherent cells. Cord blood harvests were obtained, with the mother's consent, from preterm and term deliveries at the time of birth from Hospitales Barros Luco-Trudeau and Del Salvador, Santiago. After removal of the placenta, blood was allowed to drain from the severed end of the cord into glass bottles containing 10 ml of M-199 culture medium with 250 U/ml of preservative-free heparin. In all cases, blood samples were processed ≤ 24 h after harvest. From each blood harvest, aliquots were set apart for routine haematological analysis (Cell-Dyn 3500 System, Abbott) and for immunophenotyping of haematopoietic progenitors.

Cord blood cells were separated into a low-density fraction (Hystopaque-1077; Sigma, St. Louis, USA) and mononuclear cells were washed, suspended in culture medium [α-MEM containing 20% fetal bovine serum (FBS) and

Table I. Characteristics of umbilical cord blood harvests.

Parameter	Median	Range
Gestational age (weeks)	38	32–41
WBC ($\times 10^9$/l)	13	6–22
MNCs (%)	49	31–65
HCT (%)	49	37–68
Platelets ($\times 10^9$/l)	200	67–353
$CD34^+$ ($\times 10^6$/l)	76	23–146
$CD34^+CD38^-$ ($\times 10^6$/l)	14	2·3–71
$CD34^+CD38^+$ ($\times 10^6$/l)	60	0–120

WBC, white blood cell count; MNCs, mononuclear cells; HCT, haematocrit.

80 μg/ml gentamicin sulphate; Gibco BRL, NY, USA] and seeded (T-25 flasks and 35-mm dishes) at a concentration of 1×10^6 cells/cm^2. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, with a change of culture medium every 7 d. Cells in the developing adherent layer were used for the experiments described. All determinations hereafter described were performed in the respective cell cultures originating from at least five cord blood harvests.

Cytochemical characterization. Cells *in situ* were analysed for the following cytochemical markers: acid phosphatase, alkaline phosphatase, periodic acid–Schiff, sudan black B, naphthol AS-D chloroacetate esterase and α-naphthyl

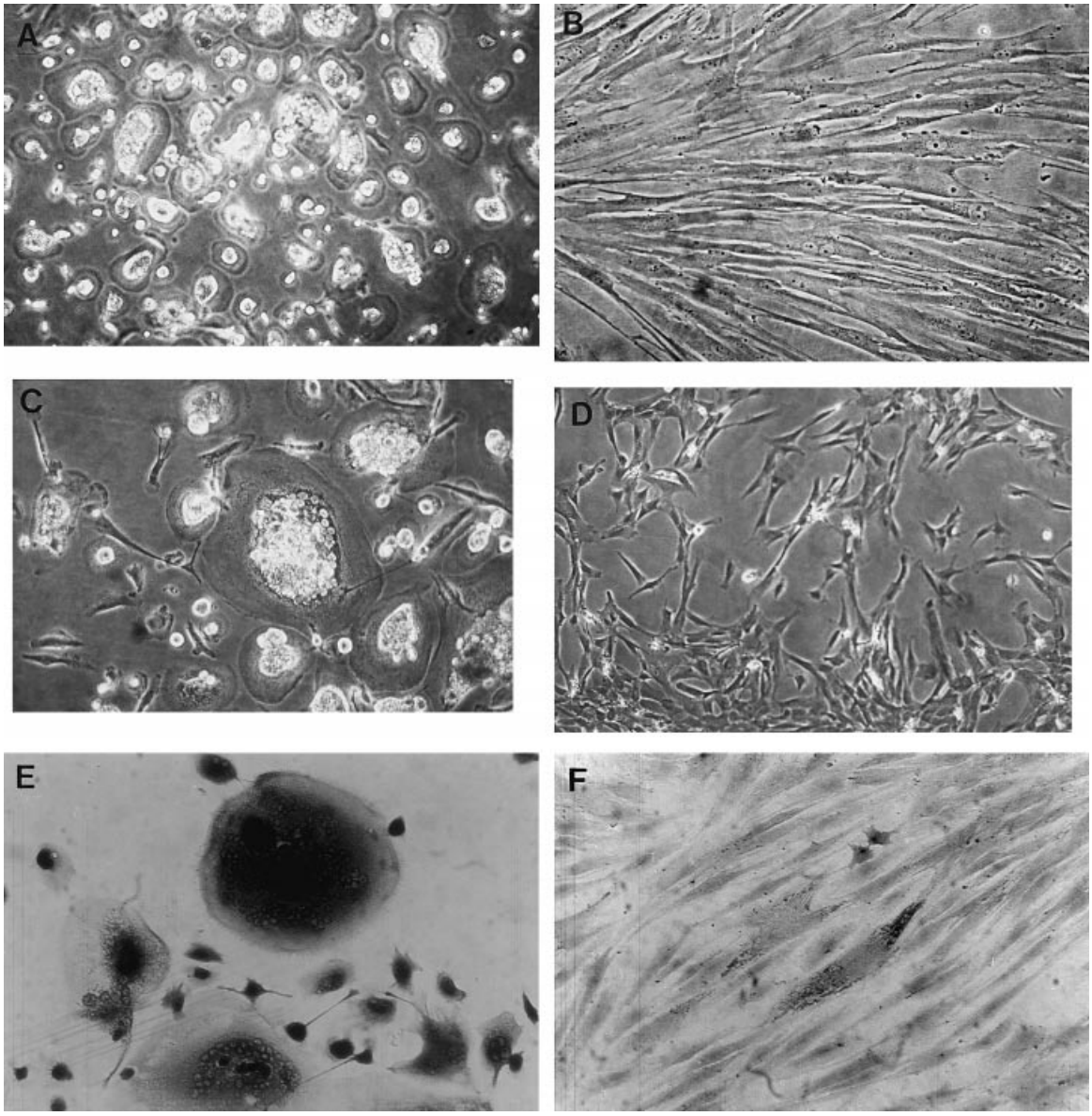

Fig 1. Photomicrographs showing adherent cells from cultures of UCB. Photomicrographs, taken after 30 d in culture, show osteoclast-like (A, C and E) and mesenchymal-like (B, D and F) cells. (A–D) Unstained cells, as visualized by phase contrast microscopy. (E and F) Cells after staining for TRAP and PAS respectively. Magnification: A and D, 100×; B, C, E and F, 200×.

Table II. Cytochemical characteristics of osteoclast-like cells (OLCs) or mesenchymal-like cells (MLCs).

Cell type	TRAP	AP	NSE	PAS	SBB	Alk.P	SE
OLCs	+	+	+	–	–	–	–
MLCs	–	–	+	+	–	–	–

Cultures containing each type of cells (day 30) were stained *in situ* for: TRAP, tartrate-resistant acid phosphatase; AP, acid phosphatase; NSE, non-specific α-naphthyl acetate esterase; PAS, periodic acid–Schiff; SBB, sudan black B; Alk.P, alkaline phosphatase; SE, specific naphthol AS-D chloroacetate esterase.

+, – indicate that ≥ 90% of cells were either positive or negative for each marker.

acetate esterase. In all cases, analyses as well as the selection of positive and negative controls were performed according to the manufacturer's guidelines (Sigma Diagnostics Kits).

Immunophenotyping of UCB or cultured adherent cells. To detect surface antigens, aliquots of fresh UCB or cultured adherent cells after detachment with 0·25% EDTA were washed with phosphate-buffered saline (PBS) containing 2% FBS. To detect intracellular antigens, cultured adherent cells were detached with 0·25% trypsin, washed with PBS and permeabilized with 70% ethanol (10 min at 4°C). For direct assays, cells were immunolabelled with the following anti-human antibodies: CD13–PE, CD31–FITC, CD54–PE, CD90–FITC, CD51/CD61–FITC (Pharmingen, Los Angeles, CA, USA), CD14–PE, CD38–FITC, CD34–PE (Dako,

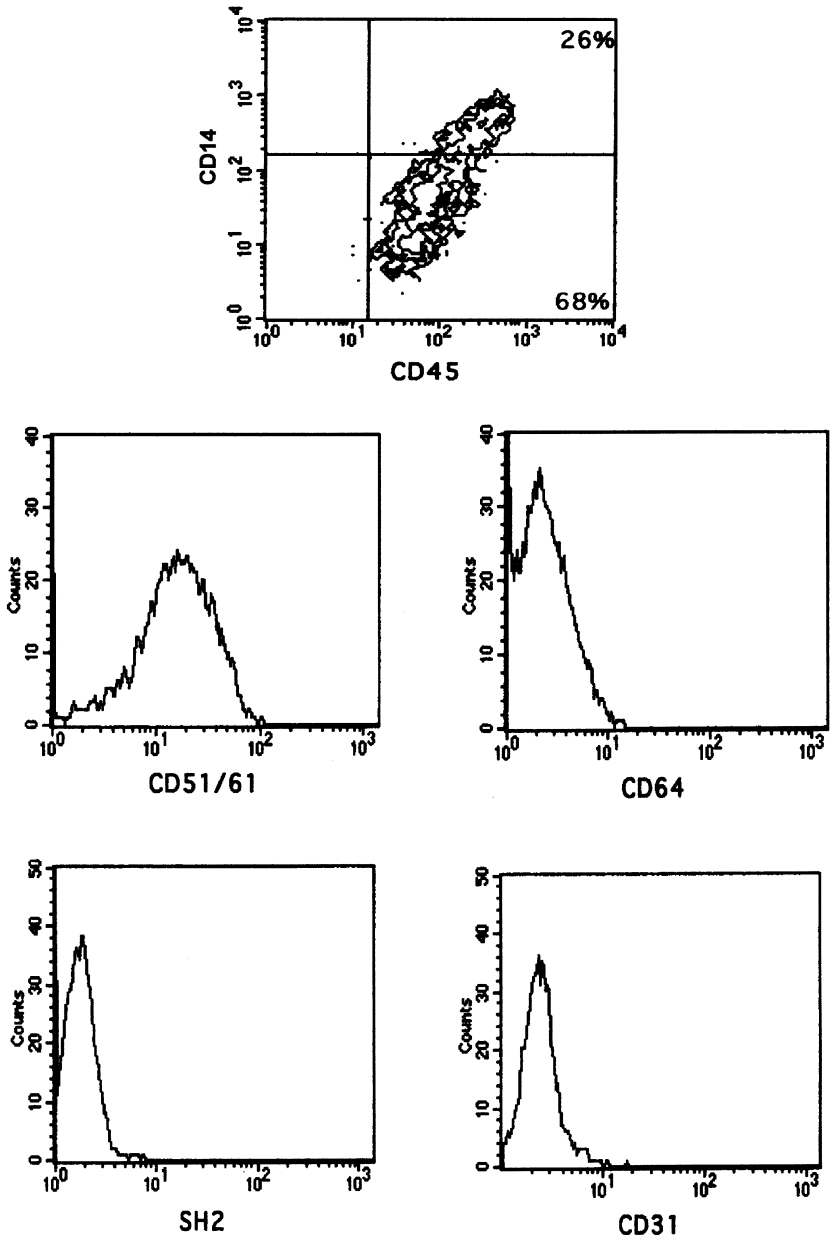

Fig 2. Immunocharacterization of osteoclast-like cells. After 5 weeks in culture, OLCs were detached with EDTA, labelled with monoclonal antibodies and enumerated by flow cytometry. Data show contour plot analysis for CD14/CD45 and histograms for CD51/CD61, CD64, SH2 and CD31 expression. Mean intensity of fluorescence of the respective control antibodies was ≤ 5 (not shown).

Glostrup, Denmark), CD29–FITC, CD45–PerCP, CD49d–PE, CD49e–FITC, CD64–FITC (Becton Dickinson, San José, CA, USA) and/or CD106–FITC (R & D Systems, Abingdon, UK). As controls, mouse IgG_1–PE, IgG_1–FITC, IgG_1–perCP or IgG_{2a}–PE (Becton Dickinson) were used. For indirect assays, cells were immunolabelled with the following anti-human antibodies: SH2, SH3, SH4 (Osiris Therapeutics, Baltimore, MS, USA), von Willebrand factor (Pharmingen), α-smooth muscle actin, ASMA (Sigma) or MAB1470 (Chemicon, Temecula, CA, USA). As secondary antibodies, anti-mouse IgG_{wm}–FITC or –PE (Sigma) were used. Labelled cells were analysed either by epifluorescence microscopy or by flow cytometry. In the latter case, 10 000 events were acquired and analysed in a FACScan flow cytometer (Becton Dickinson) using the CELLQUEST software. Note that antibodies against the antigens SH2, SH3, SH4, ASMA and MAB 1470 have been widely utilized to characterize marrow-derived mesenchymal progenitor cells (Conget & Minguell, 1999; Pittenger *et al*, 1999).

Proliferation studies. Adherent cells, after detachment with trypsin, were seeded (20×10^3 cells/ml) in culture medium. At selected time intervals, the total number of viable cells was counted and expressed as fold increase over the starting cell number, which was set to 1.

Cell cycle analysis and quantification of quiescent cells. For cell cycle analysis, cells were permeabilized and labelled with 10 μg/ml propidium iodine (Sigma), followed by treatment with 100 μg/ml RNAse A (Sigma) (Conget & Minguell, 1999). DNA content was analysed in a FACScan flow cytometer by using the MODFIT software. For assessment of quiescent cells, adherent cells were trypsinized, permeabilized and their RNA and DNA content evaluated by flow cytometry after staining with acridine orange. Under these conditions, quiescent cells (G0) are characterized by minimal RNA content and uniform DNA content (Juan & Darzynkiewicz, 1998; Conget & Minguell, 1999).

Differentiation of mesenchymal-like cells into osteoblasts and adipocytes. For these studies, cells were cultured either in an osteogenic (0·1 μM dexamethasone, 10 mM β-glycerol phosphate and 50 μM ascorbate) (Jaiswal *et al*, 1997) or adipogenic (1 μM dexamethasone, 5 μg/ml insulin, 0·5 mM isobutylmethylxanthine and 60 μM indomethacin) (Dennis *et al*, 1999) medium. The onset of osteoblasts was evaluated by the expression of alkaline phosphatase (Pittenger *et al*, 1999) or by calcium accumulation (von Kossa staining) (Jaiswal *et al*, 1997). The presence of adipocytes was assessed by the cellular accumulation of neutral lipid vacuoles that stained with Oil red O (Conget & Minguell, 1999).

RESULTS

Characteristics of cord blood harvests used for the in vitro *growth of adherent cells.*

Thirty-one cord blood harvests were entered and analysed in this study. As seen in Table I, the median values for the haematological parameters analysed were in close agreement with those reported for UCB collections for banking purposes (Beguin *et al*, 1995; Denning-Kendall *et al*, 1996). UCB from donors with a wide range of gestational ages (32–41 weeks) were included to establish whether gestational age correlates with the potential of UCB to develop adherent cells *in vitro*.

Morphological characteristics of primary cultures of UCB-derived adherent cells.

UCB-derived mononuclear cells were set in culture and the onset of an adherent layer was monitored continuously. By day 15 of culture, 29 out of 31 cord blood harvests had produced an adherent layer, which remained as such even after regular changes of the medium.

From 76% of the cord blood specimens analysed, the evolved adherent layer was formed by a heterogeneous population of cells. Based on the morphology of the most abundant phenotype present (Fig 1A and C), cells in this group of cultures were termed osteoclast-like cells (OLCs). In turn, in 24% of the UCB samples evaluated, the adherent layer contained a homogeneous population of cells showing a fibroblastoid morphology (Fig 1B and D). Cells in this

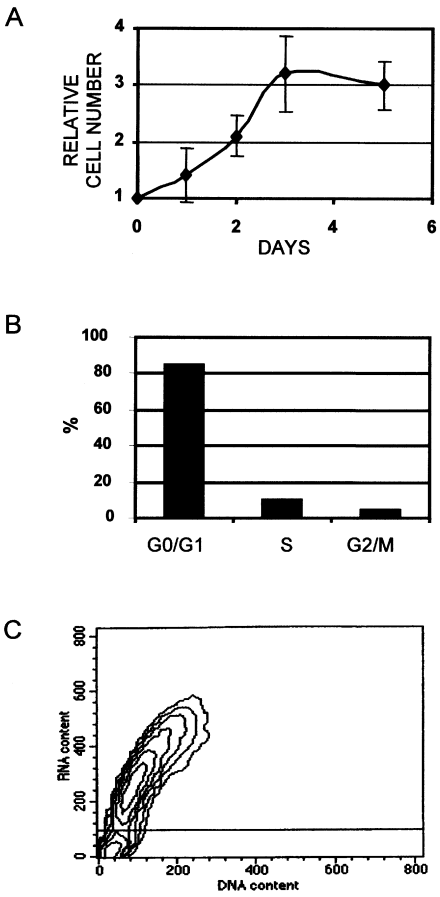

Fig 3. Proliferation and cell cycle analysis of mesenchymal-like cells. Proliferation studies (A), cell cycle analysis (B) and assessment of quiescent (G0) cells (C) were carried out as indicated in the text. For analysis of quiescent cells, quadrant determinations (not shown) were performed as previously described (Conget & Minguell, 1999).

group of cultures were termed mesenchymal-like cells (MLCs).

Characteristics of osteoclast-like cells

The morphology of OLCs was heterogeneous. Microscopic examination revealed cells with an elongated or oval/round shape with smooth borders, showing in certain cases cytoplasmic extensions. Usually, the cells were in contact with each other, however the most remarkable feature was the presence of multinucleated cells with nuclei congregated around a central area. After 3 weeks in culture, the multinucleated cells tended to predominate over the elongated and small rounded cells (Fig 1C) and reached a semiconfluent condition after 4 weeks. By subcultivation, these cells gave rise again to multinucleated cells, however their proliferation capacity was limited.

As seen in Table II, more than 90% of OLCs were strongly positive for tartrate-resistant acid phosphatase (Fig 1E), acid phosphatase and α-naphthyl acetate esterase activity, but negative for periodic acid–Schiff, sudan black B, alkaline phosphatase and naphthol AS-D chloroacetate esterase activities. As detected by flow cytometry (Fig 2), although all OLCs expressed the common leucocyte antigen CD45, only a subpopulation expressed the monocyte–macrophage antigen CD14. Together, OLCs expressed the osteoclast-related antigen CD51/CD61 (vitronectin receptor), but did

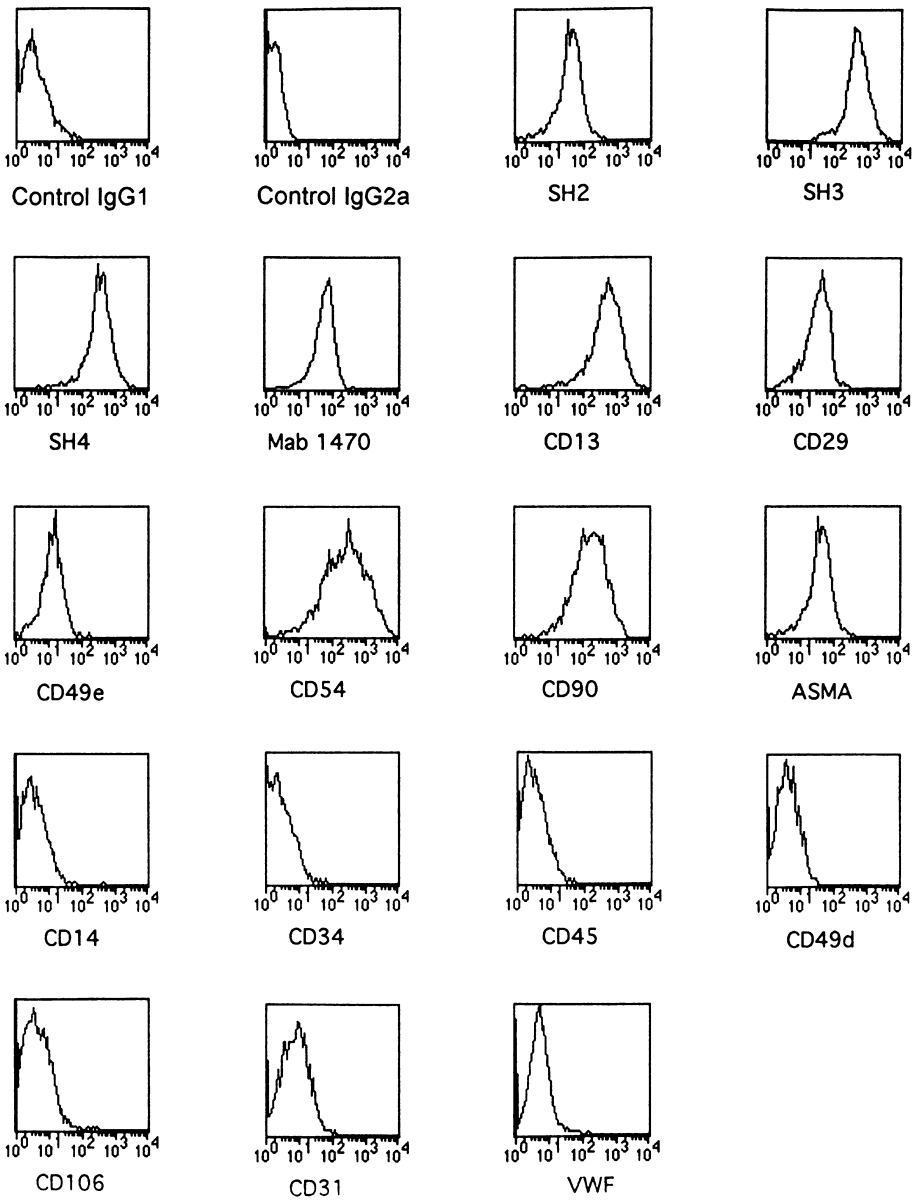

Fig 4. Immunocharacterization of mesenchymal-like cells. MLCs were detached with EDTA, labelled with monoclonal antibodies and enumerated by flow cytometry. Relative number of cells (counts) is presented vs. fluorescence intensity.

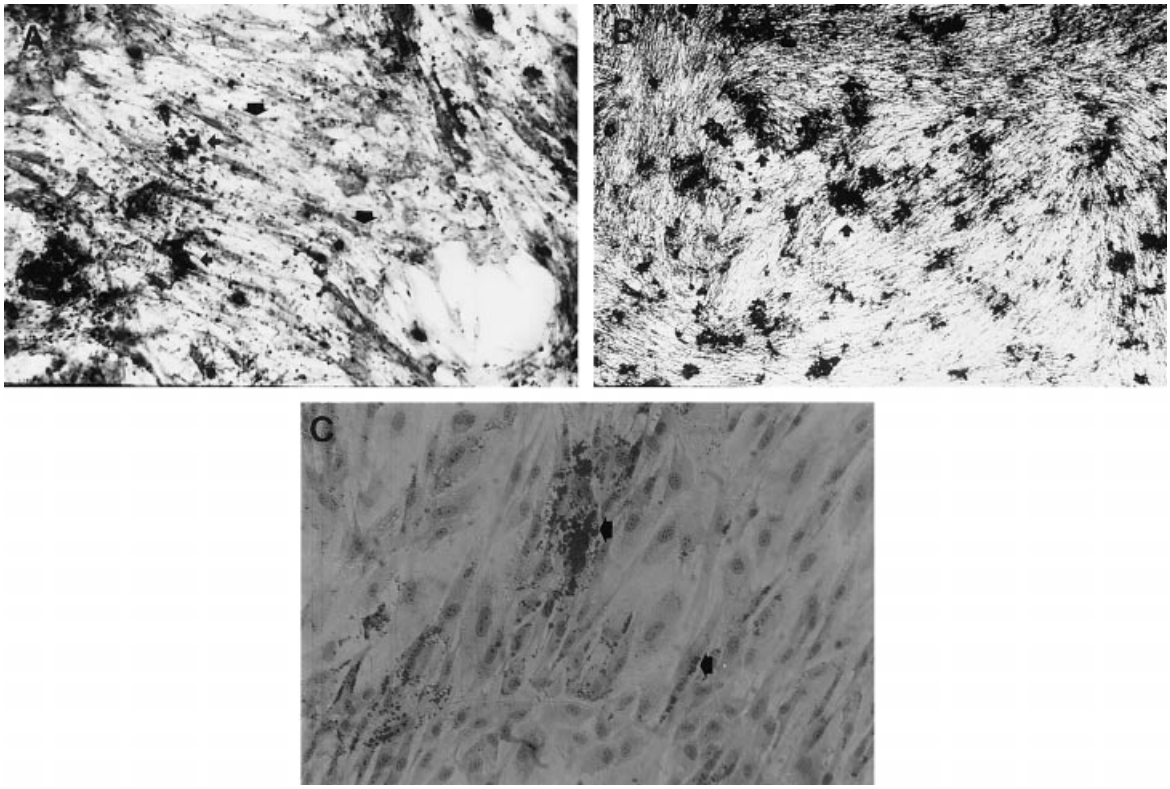

Fig 5. Differentiation potential of mesenchymal-like cells. As described in the *MATERIALS and METHODS* section, cultures of MLCs were exposed to osteogenic or adipogenic medium. Appearance of osteoblasts was detected (day 7) by either alkaline phosphatase expression (A, thick arrow) or by matrix mineralization (A and B, thin arrows). Adipocytes were detected (day 15) by accumulation of lipid drops, that stained with Oil red O (C, thick arrow). Magnification: A and C, 200×; B, 40×. Note that before the addition of the switch medium, cells appeared as shown in Fig 1D.

not express the macrophage–polykaryon (CD64)-, the mesenchymal (SH2)- or the endothelial (CD31)-related antigens (Fig 2).

Characteristics of mesenchymal-like cells

Primary cultures of MLC mainly consisted of colonies of bipolar fibroblastoid cells (Fig 1D) which, after subcultivation, proliferated with a population-doubling time of 48 h and reached a confluent growth-arrested condition (Fig 3A). In primary cultures, the MLC frequency varied from one harvest to another, however in each UCB sample the average number of MLC colonies (day 15) for 25×10^6 UCB-derived mononuclear cells was 7 ± 3.

Cell cycle analysis (at log phase of growth) indicated that $\geq$85% of the cells were in the G0/G1 phases (Fig 3B). Analysis to detect quiescent cells revealed that 5% of all MLCs displayed a pattern of RNA and DNA staining which is distinctive for G0 cells (Fig 3C).

Cytochemical analysis (Table II) revealed that MLCs were strongly positive for α-naphthyl acetate esterase and periodic acid–Schiff (Fig 1F), but were negative for all the other cytochemical markers assessed. The immunophenotype of these cells (Fig 4), as studied by flow cytometry, disclosed the homogeneous expression of the mesenchymal-related antigens SH2, SH3 and SH4. Together, MLCs were positive for an antigen (detected by MAB 1470) which although expressed by endothelial cells was also expressed by mesenchymal cells (Conget & Minguell, 1999). MLCs expressed antigens CD13, CD29, CD49e, CD54 and CD90 and α-smooth muscle actin (ASMA), but did not express antigens CD14, CD34, CD45, CD49d and CD106. In addition, MLCs did not express the endothelial-related antigens CD31 and von Willebrand factor.

Further characterization studies performed on MLCs revealed a potential to differentiate into osteoblasts and adipocytes. Thus, after switching MLCs from the regular culture medium into an osteogenic medium, cells with an osteoblast-like phenotype evolved, as judged by the expression of alkaline phosphatase (Fig 5A) and by the deposition of a mineralized matrix (Fig 5B). Moreover, when MLCs were exposed to an adipogenic medium, cells differentiated into adipocytes which displayed a perinuclear accumulation of lipid vacuoles, as detected by phase-contrast microscopy or after staining with Oil red O (Fig 5C). The above features were not observed in MLC cultures grown in regular culture medium.

DISCUSSION

Our results show that UCB-derived mononuclear cells, when cultured in a medium containing only FBS, were able to

generate adherent cells. However, we found that the nature of the adherent cells was not the same in all cases, but exhibited either an osteoclast- or a mesenchymal-like phenotype, each one characterized by the following features. First, approximately 75% of the cord blood collections gave rise to cultures of adherent cells (OLCs), which displayed the morphology and characteristics of multinucleated osteoclasts. OLCs expressed several markers of osteoclasts, such as a strong tartrate-resistant acid phosphatase activity and expression of antigens CD45 and CD51/CD61 (Udagawa *et al*, 1990; Suda *et al*, 1992). Together, these cells did not express antigens related to the macrophage–polykaryon (Quinn *et al*, 1995), mesenchymal (Conget & Minguell, 1999) or endothelial (Schwachula *et al*, 1994) lineages. In primary cultures of OLCs, we found within $CD45^+$ cells a subset that also expressed the monocyte–macrophage antigen CD14. However, the percentage of these cells decreased with cultivation time (53% and 26% at weeks 3 and 5 respectively; data not shown). The decrease in the number of $CD45^+CD14^+$ cells was coincident with the microscopic observation of a time-dependent enrichment in multinucleated cells (Fig 1C). Thus, the above characteristics strongly suggested that cells in the osteoblast-like cultures correspond to osteoclasts and/or their progenitors.

Second, almost 25% of cord blood harvests gave rise to an adherent layer, initially formed by individual cells or colonies of a few cells, which rapidly gave rise to a well-established layer of fibroblastoid (MLC) cells. The rapid growth of these cells seemed to be sustained by a population of (self-renewing?) quiescent (G0) cells. MLCs expressed several mesenchymal progenitor-related antigens, such as SH2, SH3, SH4, ASMA, MAB 1470, CD13, CD29, CD49e and CD54 (Bendall *et al*, 1993; Conget & Minguell, 1999; Pittenger *et al*, 1999). In contrast, they did not express myeloid or endothelial antigens. In addition, MLCs upon proper stimulation could be differentiated into other mesenchymal lineages, such as osteoblasts and adipocytes. Thus, in the presence of dexamethasone, β-glycerol phosphate and ascorbate, MLCs expressed bone cell markers such as alkaline phosphatase and formed mineralizing colonies (Jaiswal *et al*, 1997; Conget & Minguell, 1999). Moreover, MLCs exposed to a defined adipogenic medium for 15 d gave rise to foci of cells containing large lipid droplets that stained positively with Oil red O. Thus, the immunophenotype and functional properties displayed by cord blood-derived MLCs resembled very closely the characteristics assigned to bone marrow-derived mesenchymal progenitor cells (Galmiche *et al*, 1993; Conget & Minguell, 1999; Pittenger *et al*, 1999).

Our results showing that mature osteoclasts or their progenitors circulate in umbilical cord blood are not without precedent (Roux *et al*, 1996). However, to our knowledge, this is the first report documenting that in umbilical cord blood the presence of cells can generate MLCs upon culture. The presence of mesenchymal progenitor cells in cord blood is justified because it can be hypothesized that both haematopoietic and mesenchymal progenitors are travelling, via cord blood, from early fetal haematopoietic sites to the newly formed bone marrow (Tavassoli, 1991; Péault, 1996).

Table III. Correlation between gestational age and CD34 content of cord blood harvests with the development of cells showing either the MLC– or the OLC– phenotype.

	Type of culture* (number of cultures obtained for each type)	
Cord blood characteristics	MLC	OLC
Gestational age (weeks)		
32–36	5	3
37–41	2	19
CD34 content (%)		
$< 0{\cdot}5$	1	15
$\geq 0{\cdot}5$	6	7

* Total number of UCB analysed, 29; MLC-positive cultures, 7; OLC-positive cultures, 22.

Despite the relatively low number of UCB harvests that developed into MLCs in this study, our results suggest that preterm, as compared with term, cord blood is richer in mesenchymal progenitors, as occurs with haematopoietic progenitors (Shields & Andrews, 1998; Wyrsch *et al*, 1999). The above seems to be supported by the observed correlation between the characteristics of cord blood (gestational age and CD34 content) and the frequency of emergence of MLC-like cultures (Table III).

Based on their large *ex vivo* expansion capacity as well as on their differentiation potential, cord blood-derived mesenchymal progenitor cells can be visualized as attractive targets for cellular or gene transfer therapeutic options.

ACKNOWLEDGMENTS

A.E. thanks Diego F. Erices Contreras, his first son, for donating the most beloved UCB harvest. We thank Drs A. Pereira, J. Lira and J. Palma (Hospitales Barros-Luco Trudeau and Del Salvador, Santiago, Chile) for help with cord blood collections. We also thank Mrs. V. Simon (Clínica Las Condes, Santiago) for flow cytometry support. This work was supported by grants FONDECYT-Chile (2980017 and 8970028) and ICGEB-Italy (97/009). A.E. and P.C. are doctoral fellows from CONICYT (Chile) and Fundación Andes (Chile) respectively.

REFERENCES

Beguin, Y., Cornu, G., Brichard, B., Joris, I., Lefèvre, P., Massy, M., Paulus, J.-M., Raymakers, N., Schaaps, J.-P., Sondag, D., Van Canwerge, J.-R., Vermylen, C. & Strijckmans, P. (1995) The Belgian Cord Blood Banking Project: hematological results and practical issues (Abstract). *Blood*, **86**, 116a.

Bendall, L.J., Kortlepel, K. & Gottlieb, D.J. (1993) Human acute myeloid leukemia cells bind to bone marrow stroma via a combination of β-1 and β-2 integrin mechanisms. *Blood*, **82**, 3125–3132.

Chichester, C.O., Fernández, M. & Minguell, J.J. (1993) Extracellular

matrix gene expression by human bone marrow stroma and by marrow fibroblasts. *Cell Adhesion and Communication*, **1**, 93–99.

Conget, P. & Minguell, J.J. (1999) Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *Journal of Cellular Physiology*, **181**, 67–73.

Denning-Kendall, P., Donaldson, C., Nicol, A., Bradley, B. & Hows, J. (1996) Optimal processing of human umbilical cord blood for clinical banking. *Experimental Hematology*, **24**, 1394–1401.

Dennis, J.E., Merriam, A., Awadallah, A., Yoo, J.U., Johnstone, B. & Caplan, A.I. (1999) A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *Journal of Bone and Mineral Research*, **14**, 700–709.

Fernández, M., Simon, V., Herrera, G., Cao, C., Del Favero, H. & Minguell, J.J. (1997) Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. *Bone Marrow Transplantation*, **20**, 265–271.

Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci, E., Stornaioulo, A., Cossu, G. & Mavilio, F. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science*, **279**, 1528–1530.

Galmiche, M.C., Koteliansky, V.E., Brière, J., Hervé, P. & Charbord, P. (1993) Stromal cells from human long-term marrow cultures are mesenchymal cells that differentiate following a vascular smooth muscle differentiation pathway. *Blood*, **82**, 66–76.

Horwitz, E.M., Prockop, D.J., Fitzpatrick, L.A., Koo, W.W.K., Gordon, P.L., Neel, M., Sussman, M., Orchard, P., Marx, J.C., Pyeritz, R.E. & Brenner, M.K. (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nature Medicine*, **5**, 309–313.

Jaiswal, N., Haynesworth, S.E., Caplan, A.I. & Bruder, S.P. (1997) Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *Journal of Cell Biochemistry*, **64**, 295–312.

Juan, G. & Darzynkiewicz, Z. (1998) Cell cycle analysis by flow and laser scanning cytometry. In: Cell Biology: a Laboratory Handbook, Vol. 1 (ed. by J.E. Celis), pp. 261–274. Academic Press, San Diego.

Majumdar, M.K., Thiede, M.A., Mosca, J.D., Moorman, M. & Gerson, S.L. (1998) Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MPCs) and stromal cells. *Journal of Cellular Physiology*, **176**, 57–66.

Orkin, S. (1995) Hematopoiesis: how does it happen? *Current Opinion in Cell Biology*, **7**, 870–877.

Péault, B. (1996) Hematopoietic stem cell emergence in embryonic life: developmental hematology revisited. *Journal of Hematotherapy*, **5**, 369–378.

Pittenger, M.F., Mackay, A.M., Beck, C.B., Jaiswal, R.K., Douglas, R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Craig, S. & Marshak, D.R. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science*, **284**, 143–147.

Prockop, D.J. (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*, **276**, 71–74.

Quinn, J.M.W., Puddle, B., West, L., McGee, J. & Athanasou, N.A. (1995) Myeloid antigens on osteoclasts and macrophage polykaryons. Leucocyte Typing. V. White Cell Differentiation Antigens, Proceedings of the Fifth International Workshop and Conference held in Boston, USA, Vol. 1 (ed. by S.F. Schlossman), pp. 1012–1013. Oxford University Press, New York.

Roux, S., Quinn, J., Pichaud, F., Orcel, P., Chastre, E., Jullienne, A. & De Vernejoul, M.C. (1996) Human cord blood monocytes undergo terminal osteoclast differentiation in vitro in the presence of culture medium conditioned by giant cell tumor of bone. *Journal of Cellular Physiology*, **168**, 489–498.

Rubinstein, P., Dobrila, L., Rosenfield, R.E., Adamson, J.W., Migliaccio, G., Migliaccio, A.R., Taylor, P.E. & Stevens, C.E. (1995) Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 10119–10122.

Schwachula, A., Riemann, D., Kehlen, A. & Lagner, J. (1994) Characterization of the immunophenotype and functional properties of fibroblasts and umbilical vein endothelial cells. *Immunobiology*, **190**, 67–92.

Shields, L.E. & Andrews, R.G. (1998) Gestational age changes in circulating CD34+ hematopoietic stem/progenitor cells in fetal cord blood. *American Journal of Obstetrics and Gynecology*, **178**, 931–937.

Siena, S., Bregni, M., Brando, B., Ravagnani, F., Bonadonna, G. & Gianni, A.M. (1989) Circulation of $CD34^+$ hematopoietic stem cells in the peripheral blood high-dose cyclophosphamide-treated patients: enhancement by intravenous recombinant human granulocyte–macrophage colony-stimulating factor. *Blood*, **74**, 1905–1914.

Suda, T., Takahashi, N. & Martin, J. (1992) Modulation of osteoclast differentiation. *Endocrine Reviews*, **13**, 66–80.

Tavassoli, M. (1991) Embryonic and fetal hemopoiesis: an overview. *Blood Cells*, **1**, 269–281.

Tavassoli, M. & Minguell, J.J. (1991) Homing of hematopoietic progenitor cells to the marrow. *Proceedings of the Society for Experimental Biology and Medicine*, **196**, 367–373.

Udagawa, N., Takahashi, N., Akatsu, T., Tanaka, H., Sasaki, T., Tatsuji, N., Koga, T., Martin, T.J. & Suda, T. (1990) Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 7260–7264.

Wyrsch, A., dalle Carbonare, V., Jansen, W., Chklovskaia, E., Nissen, C., Surbek, D., Holzgreve, A., Tichelli, A. & Wodnar-Filipowicz, A. (1999) Umbilical cord blood from preterm human fetuses is rich in committed and primitive hematopoietic progenitors with high proliferative and self-renewal capacity. *Experimental Hematology*, **27**, 1338–1345.