

considerably among the cell populations derived from normal PBMNC and BM or CML at diagnosis and in stable chronic phase, a relatively constant percentage was found of adherent and nonadherent cells. The high proportion of cell loss during the experimental procedures is due to the separation technique and only to a lesser degree to mechanical damage during removal of adherent cells. Cells which were not tightly bound to plastic were discarded by washing twice with medium. However, the cell loss did not substantially affect the CFC population with regard to one-sided preference to *BCR/ABL*-positive or negative cells.

In conclusion, an accumulation was not found of *BCR/ABL*-negative CFC in the adherent fraction of PBMNC from CML patients in stable chronic phase even when starting PBMNC population already contained a certain proportion of *BCR/ABL*-negative CFC. Plastic adherence of CFC from peripheral blood seems to be unsuitable for selecting functionally identical hematopoietic cell populations.

References

- Agarwal, R., Doren, S., Hicks, B., Dunbar, C. (1995) Long-term culture of chronic myelogenous leukemia marrow cells on stem cell factor-deficient stroma favors benign progenitors. *Blood* **85**, 1306-1312.
- Boeyum, A. (1976) Isolation of lymphocytes, granulocytes and macrophages. *Scand. J. Immunol.* **5**, 9-15.
- Carella, A., Frassoni, F., Negrin, R. (1995) Autografting in chronic myelogenous leukemia: new questions. *Leukemia* **9**, 365-369.
- Carlo-Stella, C., Mangoni, L., Piovani, G., Almici, C., Garau, D., Caramatti, C., Rizzoli, V. (1991) In vitro purging in chronic myelogenous leukemia: effect of mafosfamide and recombinant granulocyte-macrophage colony-stimulating factor. *Bone Marrow Transplant.* **8**, 265-271.
- Cervantes, F., Rozman, C. (1994) Benign hematopoietic progenitors in chronic myeloid leukemia: current status and future prospects. *Ann. Hematol.* **69**, 99-105.
- Coulombel, L., Kalousek, D., Eaves, C., Gupta, C., Eaves, A. (1983) Long-term marrow culture reveals chromosomally normal hematopoietic progenitor cells in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. *N. Engl. J. Med.* **308**, 1493-1498.
- Daley, G., Goldman, J. (1993) Autologous transplant for CML revisited. *Exp. Hematol.* **21**, 734-737.
- De Fabritiis, P., Petti, M., Montefusco, E., DePropris, M., Sala, R., Bellucci, R., Mancini, M., Lisci, A., Bonetto, F., Geiser, T., Calabretta, B., Mandelli, F. (1998) BCR-ABL antisense oligonucleotide *in vitro* purging and autologous bone marrow transplantation for patients with chronic myelogenous leukemia in advanced phase. *Blood* **9**, 3156-3162.
- Dubé, I., Kalousek, D., Coulombel, L., Gupta, C., Eaves, C., Eaves, A. (1984) Cytogenetic studies in early myeloid progenitor compartments in Ph⁺-positive chronic myeloid leukemia. II. Long-term culture reveals the persistence of Ph⁺-negative progenitors in treated as well as newly diagnosed patients. *Blood* **63**, 1172-1177.
- Dunbar, C., Stewart, F. (1992) Separating the wheat from the chaff: selection of benign hematopoietic cells in chronic myeloid leukemia. *Blood* **79**, 1107-1110.
- Gordon, M., Dowding, C., Riley, G., Goldman, J., Greaves, M. (1987) Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. *Nature* **328**, 342-344.
- Grand, F., Marley, S., Chase, A., Titley, I., Healy, L., Spencer, A., Reiter, A., Goldman, J., Gordon, M. (1997) BCR/ABL-negative progenitors are enriched in the adherent fraction of CD34⁺ cells circulating in the blood of chronic phase chronic myeloid leukemia patients. *Leukemia* **11**, 1486-1492.
- Haines, M., Goldman, J., Worsley, A., McCarthy, D., Wyatt, S., Dowding, C., Kearny, L., Th'ng, K., Wareham, N., Pollock, A., Galvin, M., Samson, D., Geary, C., Catovsky, D., Galton, D. (1984) Chemotherapy and autografting for chronic granulocytic leukemia in transformation: probable prolongation of survival in some patients. *Br. J. Haematol.* **58**, 711-721.
- Heissig, B., Schultheis, B., Hochhaus, A., Pasternak, G., Hehlmann, R. (1997) A significant proportion of bcr-abl positive hematopoietic progenitors is present after long-term culture of peripheral blood mononuclear cells from patients on interferon- α therapy. *J. Mol. Med.* **75**, B247. (Abstract)
- Hogge, D., Coulombel, L., Kalousek, D., Eaves, C., Eaves, A. (1987) Nonclonal hematopoietic progenitors in a G6PD heterozygote with chronic myelogenous leukemia revealed after long-term marrow culture. *Am. J. Hematol.* **24**, 389-394.
- Hörner, S., Pasternak, G., Hehlmann, R. (1997) A statistically significant sex difference in the number of colony-forming cells from human peripheral blood. *Ann. Hematol.* **74**, 259-263.
- Lichter, P., Cremer, T. (1992) Chromosome analysis by non-isotopic *in situ* hybridization. In: *Human Cytogenetics*, eds. D. Rooney, B. Czepulkowski, pp. 157-192, Oxford University Press, New York.
- McGlave, P., Mamus, S., Vilen, B., Dewald, G. (1987) Effect of recombinant gamma interferon on chronic myelogenous leukemia bone marrow progenitors. *Exp. Hematol.* **15**, 331-335.
- McGlave, P., Arthur, D., Miller, W., Lasky, L., Kersey, J. (1990) Autologous transplantation for CML using marrow treated ex vivo with recombinant human interferon gamma. *Bone Marrow Transplant.* **6**, 115-120.
- McGlave, P., Verfaillie, C., Miller, J. (1993) Chronic myelogenous leukemia: in search of the benign hematopoietic stem cell. *Stem Cells* **11**, 10-13.
- Pasternak, G., Schultheis, B., Heissig, B., Hörner, S., Sick, C., Hehlmann, R. (1999) Does long-term culture favor normal clonogenic cells from interferon-treated patients with chronic myelogenous leukemia? *Leukemia* **13**, S55-64. (Suppl.)
- Reiffers, J., Trouette, R., Marit, G., Montastruc, M., Fabres, C., Cony-Makhoul, P., Bourdeau, M., Bilhou, X., Nabera, C., Lacombe, F., Feuillatre-Fabre, F., Vezon, G., Bernard, P., Broustet, A. (1991) Autologous blood stem cell transplantation for chronic granulocytic leukemia in transformation: a report of 47 cases. *Br. J. Haematol.* **77**, 339-345.
- Reiffers, J., Mahon, F., Boiron, J., Fabres, C., Marit, G., Cony-Makhoul, P., Broustet, A. (1996) Autografting in chronic myeloid leukemia: an overview. *Leukemia* **10**, 385-388.
- Schultheis, B., Heissig, B., Sick, C., Hochhaus, A., Pasternak, G., Hehlmann, R. (1997) Long-term cultured colony-forming cells from peripheral blood from CML patients under interferon-alpha or hydroxyurea therapy show differences in BCR/ABL positivity. *Blood* **90**, 282b. (Abstract)

Szczylik, C., Skorski, T., Nicolaides, N., Manzella, L., Mala-
guarnera, L., Venturelli, D., Gewirtz, A., Calabretta, B.
(1991) Selective inhibition of leukemia cell proliferation by
bcr-abl antisense oligodeoxynucleotides. *Science* **253**, 562-
565.

Udomsakdi, C., Eaves, C., Lansdorp, P., Eaves, A. (1992)
Phenotypic heterogeneity of primitive leukemic hemato-
poietic cells in patients with chronic myelogenous leukemia.
Blood **80**, 2522-2530.

Verfaillie, C., Bhatia, R., Miller, W., Mortari, F., Roy, V.,
Burger, S., McCullough, J., Stieglbauer, K., Dewald, G.,
Heimfeld, S., Miller, J., McGlave, P. (1992) *BCR/ABL*-ne-

gative primitive progenitors suitable for transplantation can
be selected from the marrow of most early-chronic phase but
not accelerated-phase chronic myelogenous leukemia pa-
tients. *Blood* **87**, 4770-4779.

Verfaillie, C., McCarthy, J., McGlave, P. (1992) Mechanisms
underlying abnormal trafficking of malignant progenitors in
chronic myelogenous leukemia. Decreased adhesion to stro-
ma and fibronectin but increased adhesion to basement
membrane components laminin and collagen type IV. *J.
Clin. Invest.* **90**, 1232-1241.