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WHOLE MEGAKARYOCYTES ARE PRESENT AMONG CD34+ CELLS IN THE PERIPHERAL BLOOD OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AFTER INTENSIVE CHEMOTHERAPY

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In the early '60s megakaryocyte (Mk) nuclei were identified morphologically in cell concentrates of the blood in normal individuals.[1] Thereafter, Mk nuclei or whole Mks were observed in blood of patients with marrow disorders.[2,3] More recently, a number of studies have indicated that after chemotherapy and/or hemopoietic growth factor treatment, peripheral blood progenitor cells (PBPC) contain hemopoietic precursors capable of giving rise to Mk colonies.[4,5] Here, we show that whole Mks are present among peripheral blood $CD34^+$ cells of patients with acute myeloid leukemia (AML) after intensive chemotherapy.

Patients and Methods

Heparinized blood (100 mL) was obtained after informed consent from the catheter situated in the right atrium in five patients with AML when white blood cells rose above 1.0×10^9/L after induction of remission with intensive chemotherapy (DNR, ARA-C, VP-16: 2 patients; mitoxantrone, ARA-C, VP-16: 2 patients; IDA, ARA-C, VP-16: 1 patient). Mononuclear cells were isolated by Ficoll-Hypaque density centrifugation. $CD34^+$ cell enrichment was performed via immunoadsorption using the Ceprate LC column (CellPro Inc, Bothell, WA). To assess the clonogenic capability of these peripheral blood-derived $CD34^+$ cells, an aliquot (3000 cells) was plated in methylcellulose progenitor culture for 14 days and colony forming cells (CFC) were counted.

Cytocentrifuge preparations were set up using 5×10^4 $CD34^+$ cells. Slides were stained with May-Grünwald-Giemsa and Mks were enumerated under a light microscope.

Results

A mean of 145±27 CFC/3000 $CD34^+$ cells were generated, and two to four intact Mks were identified in four out of the five patients (Figure 1). The only patient who presented neither Mks in the cytospin preparation nor CFC in the clonogenic assay showed intense marrow fibrosis, as judged by marrow biopsies performed before and after intensive chemotherapy. Marrow fibrosis could probably account for the lack of Mks in the cytocentrifuge preparation from this last patient, who did not show hemopoietic precursors, as demonstrated by the lack of CFC in the clonogenic assay.

Discussion

Blood stem cell mobilization for PBPC transplantation has been facilited by improvements in $CD34^+$ cell collection, selection and processing.[6] The rapidity of platelet recovery after PBPC transplantation has indicated that PBPC are superior to an equivalent marrow population.[7,8] Recently, Siena *et al.*[5] were able to quantify Mk progenitors circulating in the peripheral blood of patients after high-dose cyclophosphamide and recombinant hemopoietic growth factors.

The authors inferred that rapidly proliferating day-12 Mk colonies probably contain the progenitor cells responsible for rapid platelet recovery after PBPC transplantation. The identifica-

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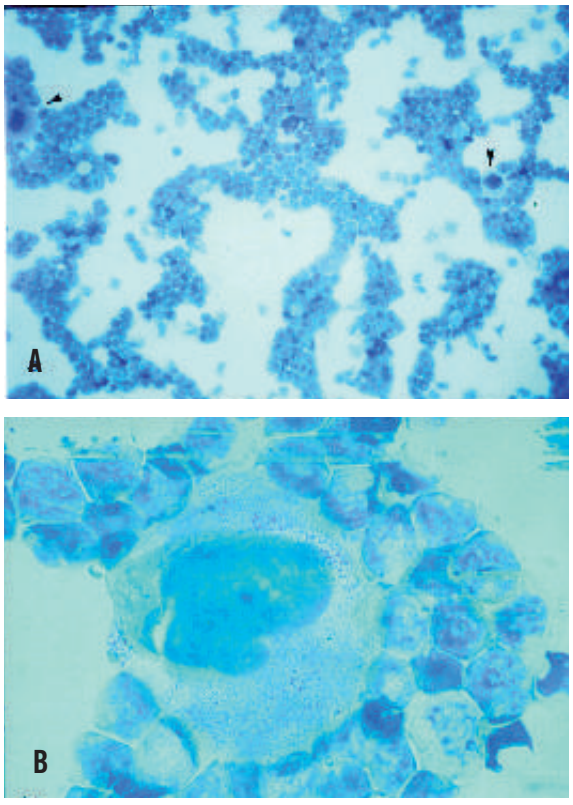

Figure 1. a) cytospin preparation of enriched peripheral blood $CD34^+$ cells. Whole Mks scattered among mononuclear cells are shown (arrows). MGG ×630; b) Higher magnification of an intact Mk surrounded by platelets. MGG ×1000.

tion of whole Mks circulating in the peripheral blood of patients after intensive chemotherapy substantiates this hypothesis. Moreover, in this brief report, intact Mks were identified in cytospin preparations after CD34 immunoselection, raising the possibility that these cells are indeed late Mk precursors still expressing the CD34 antigen.[9] Studies are in progress in order to better quantify these cells and to seek a correlation with platelet recovery time after PBPC transplantation.

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