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To The Editor:

The density distribution of rodent megakaryocytes reported by Grossi et al may enable separation of rat megakaryocytes with minimal selective depletion of particular subpopulations. However, I would like to correct a misleading statement and briefly comment on parallel considerations in the human model.

Previously, we have reported that >90% of mature morphologically recognizable human megakaryocytes,[1] as well as virtually all immature[2] and atypical myeloproliferative megakaryocytes[3] have buoyant densities ≤1,050 g Percoll per milliliter. We have also reported that ~2% of marrow cells bearing platelet glycoprotein IIb were found in gradient fractions with densities >1,050 g Percoll per milliliter. However, no efforts were made in any of these studies to characterize the density behavior of megakaryocytes in other species, and it is possible that differences between species exist. It is anticipated that multiparameter analyses by flow cytometry will provide a more accurate distributional profile of human megakaryocytes and enable the development of more efficient enrichment procedures.

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CHARACTERIZATION OF IMMATURE T CELL SUBPOPULATIONS IN NEONATAL BLOOD

To the Editor:

We have studied the in vitro differentiation of cord blood lymphocytes induced by thymic factors and hormones for some time and are interested in the recent article of Griffiths-Chu et al. These authors reported, among other differences in phenotypic markers, a lower percentage of cells positive for OKT3, OKT4, and OKT11 (Ortho Diagnostics, Raritan, NJ) in neonatal blood than in adult blood samples.[1] However, a few points in their methods used were not clear to us. The authors separated mononuclear cells from neonatal and from adult blood specimens by means of Ficoll-Hypaque, but seemed to have ignored the percentage of monocytes obtained in such samples. It is well known that cord blood or neonatal blood contains more monocytes.[2] According to our experience, mononuclear cell samples separated by Ficoll-Hypaque (Nyegaard, Oslo) from cord blood contain 21.1% ± 6.8% (mean ± SD, n = 10) of monocytes, (*v* 14.6% ± 3.9% in adult samples) as shown by α-naphthylacetate esterase staining. This might account for their (falsely) low percentage of cells positive for OKT3 and OKT11 (and, in fact, for all the other percentages given).

Taking this into consideration, and after depletion of monocytes by incubating the mononuclear samples in plastic flasks for 30 minutes, we have found no significant difference in the percentage of cells positive for OKT3 and OKT11 between cord blood and adult blood specimens, and a higher percentage of $OKT4^+$ cells in cord blood lymphocytes.[3] Similar results have been reported by other authors.[4]

The authors also alleged that lysis with 0.15 mol/L NH_4Cl and 0.01 mol/L $KHCO_3$ did not alter antigen expression. This might be true for normal adult blood samples, which they have apparently investigated. Our experience shows that, at least in cord blood lymphocytes, lysis with NH_4Cl markedly alters the percentages of cells positive for OKT3, OKT4, and OKT8, as shown in Table 1. This might again account for the lower percentages of cells positive for different antigens in cord blood samples.

We have also investigated ten cord blood samples for OKT6 but could not manage to find more than 2% of lymphocytes positive for OKT6.

Nevertheless, by studying the purine enzyme pattern and phenotypic markers in cord blood lymphocytes, we do agree with the authors that neonatal lymphocytes are immature as compared with adult lymphocytes. They suggested that these cells represent immunoincompetent cells that might differentiate in an extrathymic site. In fact, we have shown that thymosin fraction 5 and thymosin α_1 are able to induce biochemical and immunologic differentiation of

Table 1. Effect of Lysis With NH_4Cl on the Surface Antigens in Cord Blood Lymphocytes

	OKT3	OKT4	OKT8
Without lysis	77.0 ± 11.8	58.7 ± 9.4	25.6 ± 7.6
With lysis	41.0 ± 9.8	45.7 ± 10.2	16.6 ± 6.7

Results are all given as the mean percentage ± SD (n = 9).