

Letter to Editor

Cord blood lymphocytes have a low frequency of cytokine producing T cells due to a high threshold for activation

To the Editor

The use of CB for transplantation instead of bone marrow (BM) has provided a promising alternative for stem cell transplantation. Since the first successful CB transplant in 1988 [1] CB has been used for stem cell transplantation in both the related and unrelated setting. In both these settings it has been suggested that CB can cause less graft-versus-host-disease (GvHD) than BM and that a CB transplant may be able to tolerate more HLA-mismatches [2] than a BM transplant. However, these observations remain controversial and the mechanisms undefined.

Recently Chalmers et al. [3] showed that CB lymphocytes have a reduced frequency of cytokine producing cells compared with adult lymphocytes using intracellular cytokine staining [3] and suggested that this may play a role in reducing the GvHD with a CB transplant. This is a relatively new and powerful technique which measures the frequency of cells producing cytokines.

We have shown that under the same stimulation conditions that Chalmers et al. [3] used, the frequency of T cells which produce cytokines is reduced in CB compared with adult mononuclear cell population. The frequency of IL-2 and IFN γ producing T (CD3+) cells within the CB mononuclear cell population was re-

duced compared with the equivalent adult cell population ($P < 0.000001$, $P < 0.02$ for IFN γ and IL-2, respectively, in the T cells) (Table 1 is a summary of our results). Chalmers et al. [3] suggested that there is a 'defect' in intracellular signalling by CB CD45RA+ cells which prevents the lymphocytes from responding to the mitogens phorbol-12-myristate-13-acetate and Ionomycin (PMA + I). We assessed whether the inability of CB T cells to produce detectable levels of cytokine in response to PMA + I stimulation was due to the concentration of mitogen used. Because the inability of CB cells to produce cytokine has been attributed to the naiveté of the cells within CB, we focused our analysis on pure CD45RA+ (naive) T cells which had been derived from CB. Our control population of cells was a T cell line (CD45RO+) which would be able to produce cytokine due to earlier priming.

Stimulation of the naive CB T cells (CD3+ CD45RA+) by PMA + I at the concentrations used in the literature (5 ng/ml PMA and 1 μ M I; [3]) did not enable detection of IL-2 producing cells, as expected (Table 1). However, when we increased the concentration of PMA to 50 ng/ml we could detect > 10% T (CD3+) cells which made IL-2 (Fig. 1). This suggests that the 'defect' in CB signalling for cytokine production described by Chalmers et al. [3] can be overcome when higher concentrations of PMA are used. Thus, the low frequency of cytokine producing naive T cells derived from cord can be enhanced with high concentrations of PMA and I. We suggest that in vivo naive T

Table 1
Comparison of the ability of CB and adult T cells to make IL-2 and IFN γ on stimulation with phorbol-12-myristate-13-acetate (PMA) plus Ionomycin (I)^a

	Mean percentage of CD3+ gated cells within adult ($n = 10$) PBMC which stained for cytokine (\pm S.D.)	Mean percentage of CD3+ gated cells within CB ($n = 10$) which stained for cytokine (\pm S.D.)	<i>P</i> value (comparing adult and CB values)
IL-2	35.3 \pm 13.0	19.6 \pm 12.2	0.013
IFN γ	82.9 \pm 9.6	42.9 \pm 14.1	0.000008

^a Mononuclear cells derived from CB or adult blood were placed in stimulation mixture (20 ng/ml phorbol-12-myristate-13-acetate, 1 μ M Ionomycin, 3 μ M Monensin and 10% human serum; PMA+I), for 6 h at 37°C in 5% CO₂. Cells were then stained extracellularly for CD3 and intracellularly for cytokine [4]. T cells (CD3+) were analysed for their ability to produce the cytokines IL-2 and IFN γ . *P* values were determined by comparing the percentages of T cells which made IL-2 after stimulation in the adult and CB samples using a two-tailed Student's *t*-test. Results were assumed significant if $P < 0.05$.

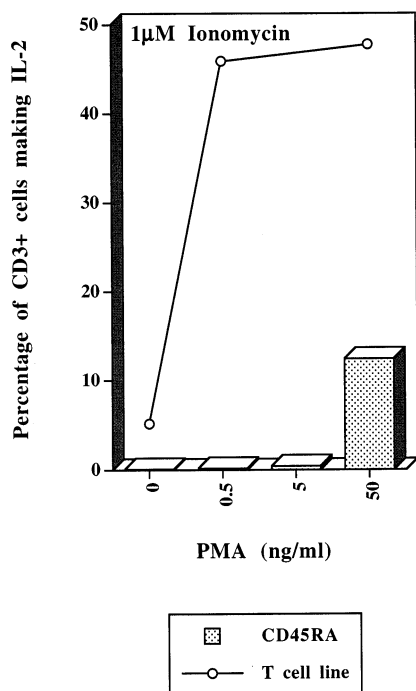


Fig. 1. The ability of CB naive (CD45RA+) T cells to make IL-2 can be enhanced by increasing concentrations of phorbol-12-myristate-13-acetate (PMA). Pure CD45RA+ cells derived from CB and a positive control population (T cell line) were stimulated for 6 h with 1 μ M I plus varying concentrations of PMA. All stimulations were performed in the presence of Monensin. Control wells were left without stimulation. Cells were then stained extracellularly for CD3 and intracellularly for IL-2, as described [4]. Results are expressed as the percentage of T cells (CD3+) which were stained by the anti-IL-2 antibody above the isotype matched negative control antibody level staining. This figure is a representative experiment of two experiments.

cells derived from cord blood may not have the ability to make cytokine due to a high threshold for activation and not an intrinsic defect, and this may explain the

reduced GvHD when CB is used for stem cell transplantation.

References

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