LETTER TO THE EDITOR

Does Nitric Oxide Generated by Dendritic Cells Contribute to the Low Incidence of GVHD after Cord Blood Transplantation?

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Cord Blood Transplantation (CBT) has a significantly lower incidence of graft-versus-host disease (GVHD) compared to marrow or peripheral blood transplantation (1). There are largely unknown T-cell silencing factors involving in the less severe GVHD after CBT. Sorg et al. highlighted the lower functional capacity of the Cord Blood Dendritic Cells (CBDCs) (2). The available evidence shows that there are a multitude of differences between CBDCs and PBDCs: CBDCs are in general functionally immature with low expression of costimulatory molecules, deficient in interleukin-12 (IL-12) production, less effective in stimulating T-cell proliferation, and therefore favoring immune tolerance (3). In this study, we have approached the low risk of GVHD in CBT from a new angle by focusing on DC-derived Nitric Oxide (NO) which has recently been shown to inhibit T cell proliferation and induce T cell apoptosis (4). We designed experiments to determine whether an increase in NO synthesis in CBDCs plays a protective role in severe GVHD and evaluated NO production in Mixed Leukocyte Reaction (MLR) using pure populations of CBDCs and PBDCs. A total of 14 samples of fresh CBDCs and PBDCs were isolated as lineage-negative cells according to a slightly modified protocol described previously (5). Using a cocktail of PE conjugated anti-lineage markers (CD3, CD19, CD34, CD16, CD56, CD14, CD11b, and CD66b), FITC conjugated anti-HLA-DR monoclonal antibodies, and flow cytometric analysis, the frequency of Lin- HLA-DR cells (DCs) was determined to be 58.5 ± 4.5 % and 45 ± 12 % of enriched cells from PB and CB, respectively.

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For estimation of NO in culture supernatants of pure DC population, $5 \times 10^5$ samples of enriched CBDCs or PBDCs were incubated in both the presence and absence of lipopolysaccharide (LPS) (10 µg/ml; *Escherichia coli* 026:B6, Sigma-Aldrich, St Louis, MO, USA) in a final volume of 200 µl in round-bottom 96-well plates for 48 h at 37°C. For estimation of NO in culture supernatants MLR, $10^5$ PB and CBDCs cells were co-cultured with $10^6$ allogeneic T cells in a final volume of 200 µl in round-bottom 96-well plates for 5 days. Clear supernatants were collected and the levels of NO were measured by gries reaction (6).

Our results showed that CBDCs and PBDCs both produced significant amounts of NO spontaneously (Figure 1). The NO production was clearly augmented after LPS stimulation as well as allogeneic T cells co-culture (Figure 2). However, our results did not show superior potency of CBDCs in NO production compared with PBDCs.

**Figure 1.** Spontaneous nitric oxide production by pure populations of CB and PB dendritic cells.

**Figure 2.** Nitric Oxide production of CB and PB dendritic cells in allogeneic MLR.
Although it is documented that NO is associated with low stimulatory capacity of DC (4) and GVHD-associated immunosuppression (7), our results do not support the possibility that the reported protective role of CBDCs in severe GVHD [such as impairment of T-cell proliferation (2), apoptotic deletion of antigen specific T cells, (8) and biased immune responses against Th1 (9)] are attributed to the ability of CBDCs in NO production.

REFERENCES