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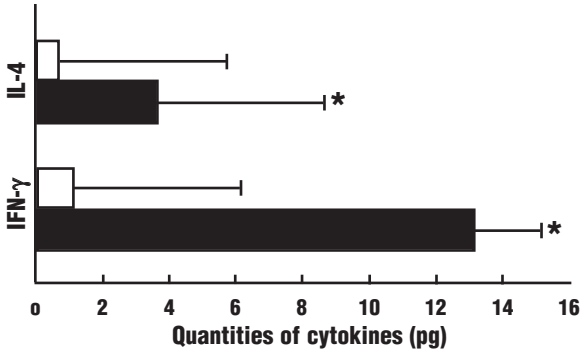

Figure 1. The quantities of type 1 (IFN-γ) and type 2 (IL-4) cytokines secreted by lymphocytes per microliter between G-PB and G-BM, the formula for the calculation of the cytokines as follows: quantities of cytokines=secretion of cytokines (pg/10^6 MNCs)×cell counts (MNCs/μL). *$p<0.001$, *independent t-tests was used (G-PB vs G-BM).*

Stem Cell Transplantation

A direct comparison of immunological characteristics of granulocyte colony-stimulating factor (G-CSF)-primed bone marrow grafts and G-CSF-mobilized peripheral blood grafts

Our preliminary results suggest the existence of quantitative and qualitative differences in immune cells and type1 and type2 cytokines between granulocyte colony-stimulating factor (G-CSF) primed bone marrow (G-BM) and G-CSF-mobilized peripheral blood grafts (G-PB). Our findings suggest that lower T-cell hyporesponsiveness and easier polarization of T cells from Th1 to Th2 are found in G-BM.

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Preliminary clinical trials have indicated that G-BM results in comparable engraftment, reduced severity of acute graft-versus-host disease (GVHD), and less subsequent chronic GVHD, as compared with G-PB.[1,2] Moreover, G-BM transplantations produce even less chronic GVHD than do steady-state bone marrow grafts.[2] In this study, we report on the immunological cells and the type1/type2 cytokine profile of lymphocytes present in G-PB and G-BM harvests.

The donors, consisting of eight men and seven women, provided informed consent and ranged in age from 13 to 65 years, with a median age of 40 years. Approval for this study was obtained from the Institutional Review Board and Ethical Committee of the Health Center at Peking University. Samples of G-BM and G-PB were obtained, isolated, and evaluated as described previously.[3,4] It was ensured that the G-BM and G-PB had the same cell concentration. Statistical comparisons were performed using t-tests for independent samples. The lymphocyte proliferation ability in G-PB (stimulation index: 1.13±0.24) was significantly higher than in G-BM (0.98±0.14, p=0.045; n=15 experiments). This finding suggests hyporesponsiveness of T cells in G-BM and is likely related to the lower incidence of GVHD observed in G-BM transplants.

The quantities of interferon-γ (IFN-γ) (13.19±14.33 pg) and interleukin-4 (IL-4) (3.67±1.77 pg) secreted per microliter of G-PB mononuclear cells were, respectively, 8.5- and 4.5-fold higher than those of G-BM mononuclear cells (1.31±0.57 pg and 0.75±0.24 pg; p<0.001) (Figure 1). The ratio of IL-4/IFN-γ was significantly lower in G-PB than in G-BM (0.33±0.23 vs. 0.73±0.16, p<0.001). These results suggest that bone marrow T cells could be easily polarized from Th1 to Th2 and that patients transplanted with G-PB could accept more type1/type 2 cytokines than could patients transplanted with G-BM. The type-1 to type-2 immune deviation after *in vivo* application of G-CSF is associated with decreased acute GVHD or with the development of a *chronic* GVHD syndrome, characterized by decreased mortality and autoantibody formation.[5]

Krenger and Ferrara have proposed a model in which type 1 cytokines (IL-2, IFN-γ) are involved in the physiopathology of acute GVHD, and type 2 cytokines (IL-4, IL-10) play a crucial role in the physiopathology of chronic GVHD.[5] Fowler *et al*. found that type 1 and type 2 cells appear to play different roles in mediating GVHD and graft-versus-leukemia (GVL) effects.[6,7] Furthermore, type 2 T cells are more resistant to CD95 (Fas)-dependent activation-induced cell death than are type 1 T cells.[8] Therefore, the high quantities of type 1 and type 2 cytokines in G-PB may be related to the different outcomes of the GVL effect and GVHD after G-PB and G-BM transplantation.[1,2]

The quantities of nucleated cells and monocytes in G-PB were, respectively, 4- and 43-fold higher than in G-BM harvests (p<0.001), all lymphocyte subsets exhibited 26- to 46-fold higher cell counts (p<0.001), and the CD4/CD8 ratio was also significantly higher in G-PB than in G-BM (1.59±0.53 vs. 0.91±0.29, p<0.001). These findings indicate that patients transplanted with G-PB may accept more T cells and monocytes than patients transplanted with G-BM (*clinical data not shown*). The cell counts of dendritic cell (DC) 1 and DC2 subgroups in G-PB were, respectively, 11- and 7-fold higher than those in G-BM (p<0.001 and

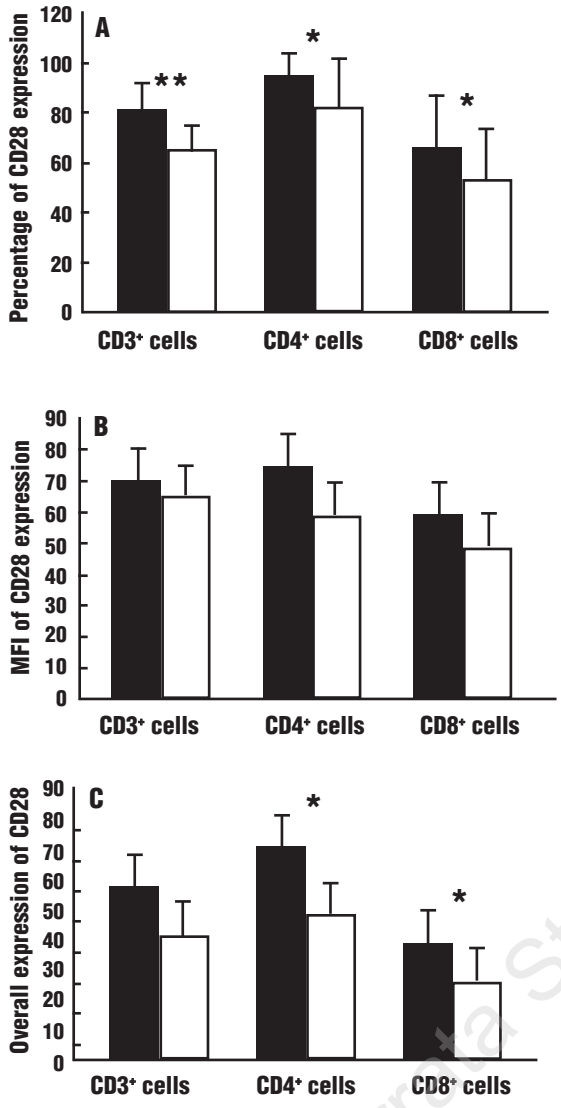

Figure 2. Expression of CD28 co-stimulatory molecules on T cell subgroups (CD3, CD4, CD8 positive cells). Percentage (A), mean fluorescence intensity (MFI) (B) and overall expression (C) on T cells of G-PB (dark boxes) and G-BM (light boxes) treatment with rhG-CSF (5μg/kg/d). *p< 0.05, **p< 0.01

p=0.001; respectively); however, the DC2:T lymphocyte ratio was significantly lower in G-PB (0.91±0.77%) than in G-BM (3.02±2.65%) (p=0.006). The percentage of CD28 expression in $CD3^+$, $CD4^+$, and $CD8^+$ T cells was lower in G-BM than in G-PB (p=0.005, 0.014, and 0.012, respectively) (Figure 2A), as was the overall expression of CD28 in $CD4^+$ and $CD8^+$ T cells (p=0.031 and 0.016, respectively) (Figure 2C). Some researchers have suggested that a modulation of T-cells from a Th1 to Th2 phenotype in G-CSF-treated bone marrow or peripheral blood harvests may be involved in the selective increase of type 2 DC and monocytes and the downregulation of CD28/B7 co-stimulatory signals.[3,4,9]

Hardling *et al*. have shown that T-cells anergy can be induced if the CD28/B7 co-stimulatory path is blocked;[10] therefore, it should be investigated whether the lower DC2:T lymphocyte ratio in G-PB and the lower expression of CD28 in G-BM $CD4^+$ and $CD8^+$ T cells contribute to the difference in polarization from Th1 to Th2 and T cell hyporesponsiveness between G-PB and G-BM.

In conclusion, we showed that there are both quantitative and qualitative differences of immunological cells and type 1 and type 2 cytokines between G-PB and G-BM. The correlation between our experimental results and the occurrence and severity of acute GVHD and chronic GVHD, relapse, or other transplant-related complications, are presently being elucidated.

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Key words: bone marrow grafts, G-CSF, peripheral blood stem cell grafts, T cells, dendritic cells, costimulatory molecules.

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