Efficient isolation of human CD34⁺CD38⁻ hematopoietic stem cells using immunomagnetic sorting

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Introduction

Hematopoietic stem and progenitor cells (HSPCs) are an attractive source for cell-based therapies. CD34⁺ cells are the main source for functional *in vitro* assays, although the majority of the CD34⁺ cells are rather progenitor than stem cells. In contrast, the CD34⁺ cell subpopulation with low CD38 expression (CD34⁺CD38⁻ cells) is highly enriched for stem cells[1] and thus much more suitable for functional analysis of stem cells, e.g., in expansion protocols and engraftment assays. Also, the more primitive CD34⁺CD38⁻ cells are being discussed as an alternative cell source for stem cell–based gene therapy approaches with the potential to improve transduction protocols[2,3].

Results

1 An easy two-step immunomagnetic protocol for isolation of CD34⁺CD38⁻ cells

CD34⁺CD38⁻ cells were isolated from fresh human cord blood by magnetic cell separation (MACS® Technology) using the CD34⁺CD38⁻ Cell Isolation Kit. CD34⁺CD38⁻ cells were isolated in a two-step protocol. First, CD34⁺ cells were positively enriched using CD34 MultiSort MicroBeads. Subsequently, the MultiSort MicroBeads were enzymatically released from the CD34 antibody. The isolated CD34⁺ cells were then labeled with CD38 MicroBeads to deplete CD38⁺ cells in a second separation step (fig. 1A). Cells were fluorescently stained using CD34, CD38, and CD45 antibodies, Labeling Check Reagent (LC), as well as propidium iodide to exclude dead cells from analysis. Cell number and immunophenotype were analyzed by flow cytometry using the MACSQuant® Analyzer. Labeling Check Reagent is used for reliable detection of CD38⁺ cells after their separation as binding of available CD38 antibody clones is sterically hindered by MicroBead labeling. The developed isolation protocol allows the separation of the two CD34⁺ cell subpopulations (fig. 1B). Starting from 1×10^8 mononuclear cord blood cells (n = 7) with a CD34⁺ cell frequency from 0.1% to 1%, we isolated 8.0×10^3 to 1.1×10^5 CD34⁺ cells with low CD38 expression and a mean CD34⁺ cell purity of 85% (range 63% to 94%). The number of CD34⁺ cells with a high CD38 expression ranged from 7.4×10^4 to 5.0×10^5 cells and a mean CD34⁺ cell purity of 82% (range 47% to 96%) was measured.

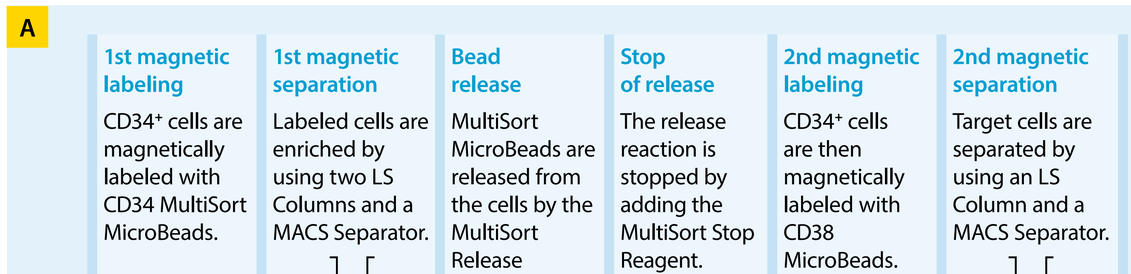

Figure 1

2 Isolated CD34⁺CD38⁻ cells remain more primitive than CD34⁺CD38⁺ cells during cultivation

The isolated CD34⁺CD38⁻ and CD34⁺CD38⁺ cells from three different donors were cultivated in StemMACS™ HSC Expansion Media XF supplemented with StemMACS HSC Expansion Cocktail for six (donor 3) or seven days (donor 1 and 2). After cultivation, the frequency of CD34⁺ cells was up to 28% higher when the culture was started with the more primitive CD34⁺CD38⁻ cells (fig. 2A). For CD133 expression this effect was even stronger as the proportion of CD133⁺ cells within the CD34⁺ cell population was up to 42% higher after cultivation of CD34⁺CD38⁻ cells (fig. 2B). Figure 2C shows a comparison of the CD34 and CD133 marker expression on the cultivated CD34⁺CD38⁻ and CD34⁺CD38⁺ cells for donor 2. In summary, cultivated CD34⁺CD38⁻ cells maintain a more primitive immunophenotype than CD34⁺CD38⁺ cells.

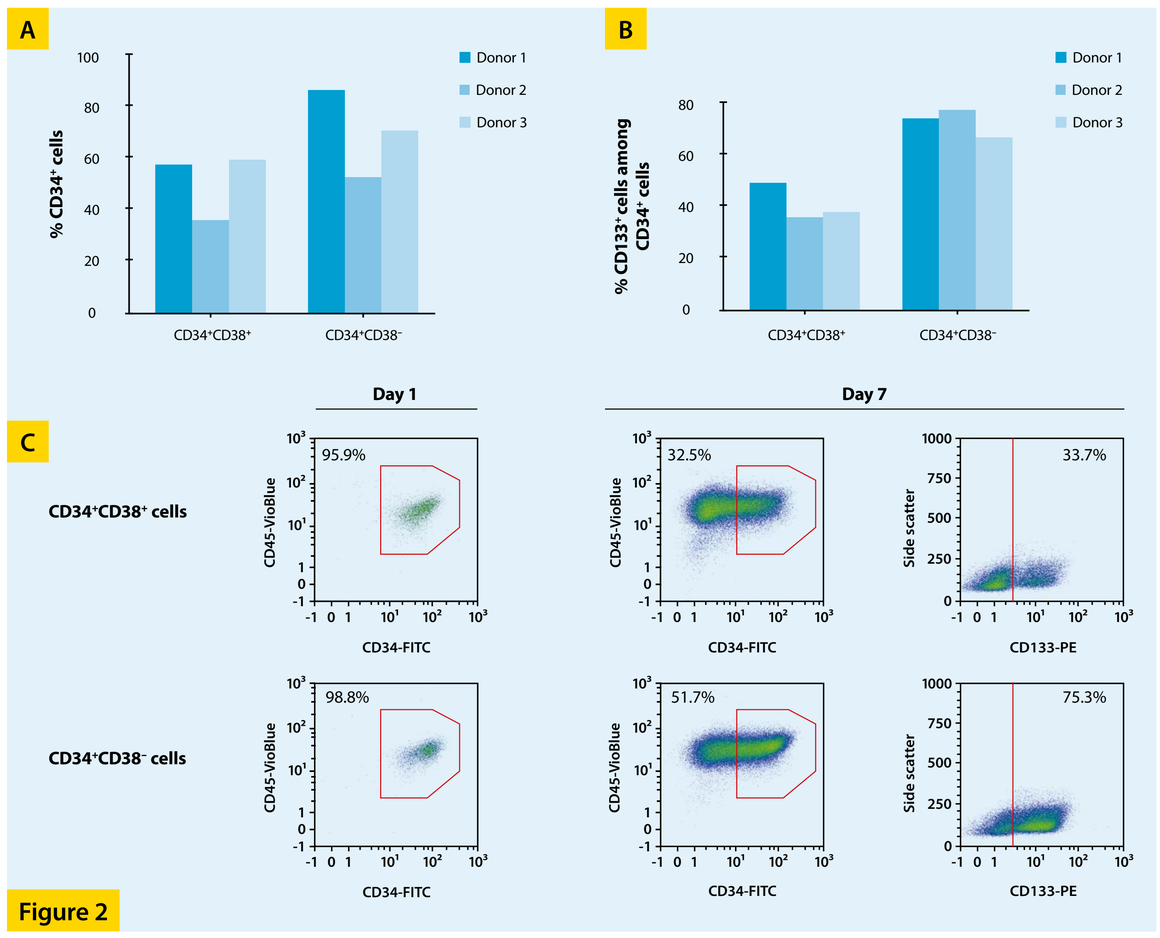

Figure 2

3 The expansion rate of CD34⁺CD38⁻ cells is higher compared to CD34⁺CD38⁺ cells

The isolated CD34⁺ cell subpopulations were also analyzed for their expansion rate during cultivation in 96-well plates. Expansion was calculated based on the number of cells seeded at day 1 and the number of cells obtained after six (donor 3) or seven days (donor 1 and 2). Cell numbers were determined by flow cytometry after gating on viable CD34⁺ cells (fig. 3A) or the more primitive CD34⁺CD133⁺ cells (fig. 3B). The expansion rate of CD34⁺ and CD34⁺CD133⁺ cells was higher with CD34⁺CD38⁻ cells compared to CD34⁺CD38⁺ cells and the difference was more pronounced for CD34⁺CD133⁺ cells. Simultaneous analysis of immunophenotype and cell divisions with carboxyfluorescein diacetate N-succinimidyl ester (CFSE) will be performed in upcoming experiments. This analysis will provide more insight into the behavior of the primitive CD34⁺CD38⁻ cells during *in vitro* cultivation.

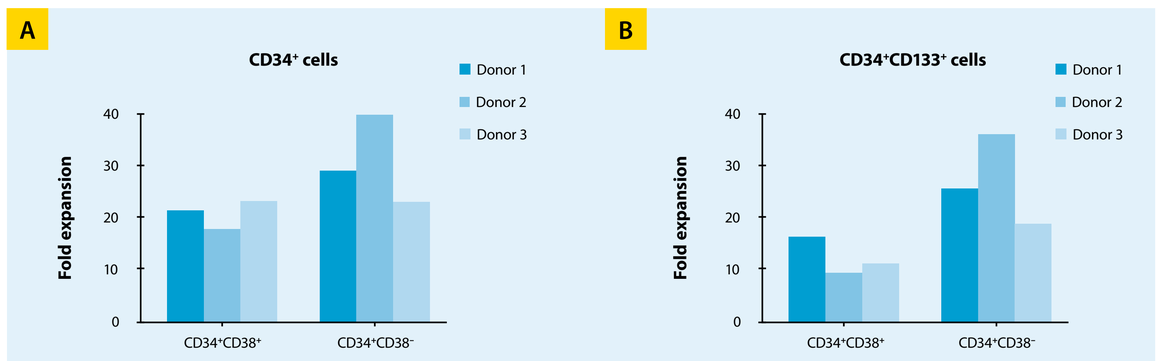

Figure 3

4 Colony-forming unit potential of CD34⁺CD38⁻ and CD34⁺CD38⁺ cells

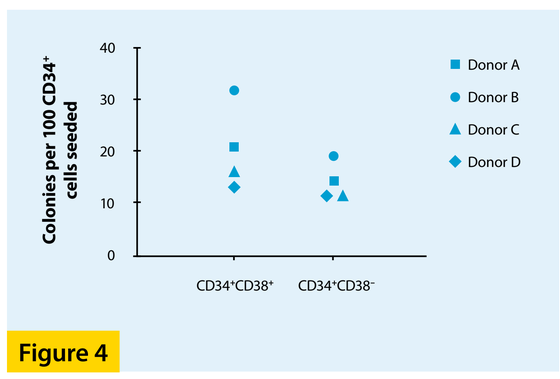

Figure 4

Isolated CD34⁺CD38⁻ and CD34⁺CD38⁺ cells from four different donors were seeded in methylcellulose medium and cultured for 14 days (fig. 4). Subsequently, cultures were analyzed microscopically and the colonies were counted as well as classified. A slightly higher colony count was observed for the CD38⁺ subpopulation. The outcome for the different colony types that were classified as burst-forming unit erythrocyte (BFU-E), colony-forming unit granulocyte (CFU-G), macrophage (CFU-M), or granulocyte-macrophage (CFU-GM) was very similar for both subpopulations analyzed.

Conclusion

- An easy two-step immunomagnetic isolation protocol for CD34⁺CD38⁻ cells was developed.
- Functional analysis of the isolated cells confirmed the more primitive phenotype of the CD38⁻ subpopulation.
- CD34⁺CD38⁻ cells maintain a more primitive immunophenotype than CD34⁺CD38⁺ cells during cell expansion.

References
1. Reems, J.A. and Torok-Storb, B. (1995) Blood 85: 1480–1487.
2. Baldwin, K. *et al.* (2015) Stem Cells 33: 1532–1542.
3. Zonari, E. *et al.* (2017) Stem Cell Reports 8: 977–990.