Stable mixed double donor chimerism
Absence of war doesn’t necessarily mean peace

Jens Gertow,* Jonas Mattsson J and Michael Uhlin*
Center for Allogeneic Stem Cell Transplantation and Division of Clinical Immunology; Karolinska Institutet; Stockholm, Sweden

Double cord blood transplantation has successfully been introduced to remedy the obstacle of a limited stem cell dose in a single cord blood graft. After a short initial period, the sustained hematopoiesis is derived almost exclusively from one of the donated units. In a recent publication in Clinical and Experimental Immunology we investigated two rare individuals in which both cord blood units co-existed for more than two years after transplantation.

So what happens with the patient’s immune system in the absence of an apparent initial conflict between the cord blood units? In a recent publication in Clinical and Experimental Immunology, we investigated two patients in which both cord blood units co-existed for more than two years after transplantation. From an international perspective these patients are extremely rare, but at our center they have been observed at quite a high frequency. Three out of seven evaluable DCBT have presented with a stable mixed donor-donor chimerism for more than three months, and when this was written a fourth patient showed the same kind of tolerance between units still six months after DCBT. In our recent paper we have thoroughly characterized two of these patients by flow cytometry.

We speculate that tolerance between the two cord blood units after stem cell transplantation (SCT) at our center develops due to: (1) a high-dose anti-thymocyte globulin (ATG) and (2) a complete donor unit match of the NK cell receptor ligands, HLA-C. ATG is used to avoid graft versus host disease and works by depleting the graft of T cells in vivo. As T cells after cord blood transplantation both reconstitute more slowly compared to adult stem cell sources and usually are present at a much lower overall number, the addition of a high-dose ATG will greatly reduce the potential for T cell mediated rejection in any direction for a prolonged time. The lack of T cells allows for the NK cells to expand more freely, an event that in an HLA-C mismatched situation could lead to unit rejections. In our situation this potential for unit rejection has also been at least partly eliminated because of the donor-donor HLA-C match.

Back to the original question: what happens with the immune system(s) post SCT of patients that have two units co-existing? This question contains many sub-categories: Will the patients have two...
equally functional immune systems? Will the T cell receptor (TCR) repertoires be twice as wide? Could it be advantageous to be a double-chimera, and therefore, is this something we should strive for?

The answer to all questions is no. The two units had comparable TCR repertoires and more importantly, they were not equally functional. Both patients presented with a major unit, taking up a larger part of the total immune system, and with T cells and NK cells responding to stimuli in a manner similar to an immune system developed after single cord blood transplantation. In contrast, the minor unit was more non-responsive and accordingly had a more naïve T cell phenotype. Consequently the two systems altogether had a more naïve phenotype and a less responsive functionality compared to single unit cord blood controls.

Apparently the two immune systems have been successful to different degrees in repopulating their new host. We speculate that the major unit indeed has developed a reaction towards the minor unit. The minor unit would by this reaction be kept in check and not allowed to expand resulting in a cold war between the units.

Even if this speculation is wrong, the minor unit still just continues to exist as an adjunct in the recipient. Thus, with our data in mind, it is unclear whether it is recommended to strive for double donor chimerism. On the other hand, one of the patients in the study is still without complications, 50 months after transplantation.

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References
1. Majhail NS, Brunstein CG, Wagner JE. Double umbilical cord blood transplantation. Curr Opin Immunol 2006; 18:571-5.
2. Ballen KK, Spitzer TR, Yeap BY, McAfee S, Dey BR, Attar E, et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. Biol Blood Marrow Transplant 2007; 13:82-9.
3. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood 2005; 105:1343-7.
4. Brunstein CG, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood 2007; 110:3064-70.
5. Guttmad JA, Turtel CJ, Manley TJ, Haimfeld S, Bernstein ID, Riddell SR, et al. Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8$^+$ T-cell response against the nonengrafted unit. Blood 2010; 115:757-65.
6. Vermeer MR, Brunstein CG, Barker J, MacMillan ML, DeFor T, McKenna DH, et al. Relapse risk after umbilical cord blood transplantation: enhanced graft-versus-leukemia effect in recipients of 2 units. Blood 2009; 114:4293-9.
7. Gerstor J, Berglund S, Okas M, Ursenel M, Berg L, Karre K, et al. Characterization of long-term mixed donor-donor chimerism after double cord blood transplantation. Clin Exp Immunol 2010; 162:146-55.
8. Yen HJ, Chiu Tj, Hung GT, Chang CY, Huieh MY, Tseng CH, et al. Long-term mixed full-donor chimerism with dominance reversion after a double-unit cord blood transplant. Eur J Haematol 2008; 80:366-7.
9. De Lima M, St. John LS, Wieder ED, Lee MS, McMannis J, Karandish S, et al. Double-chimerism after transplantation of two human leukocyte antigen mismatched, unrelated cord blood units. Br J Haematol 2002; 119:773-6.
10. Barker JN, Weisdorf DJ, Wagner JE. Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. N Engl J Med 2001; 344:1870-1.
11. Berglund S, Okas M, Gertow J, Uhlin M, Mattsson J. Stable mixed donor-donor chimerism after double cord blood transplantation. Int J Hematol 2009; 90:526-31.
12. Rembrerger M, Svaln BM, Mattsson J, Ringden O. Dose study of thymoglobulin during conditioning for unrelated donor allogeneic stem-cell transplantation. Transplantation 2004; 78:122-7.
13. Ringden O, Okas M, Uhlin M, Ursenel M, Rembrerger M, Mattsson J. Unrelated cord blood and mismatched unrelated voluntary donor transplants, two alternatives in patients who lack an HLA-identical donor. Bone Marrow Transplant 2008; 42:645-8.
14. Willemze R, Rodrigues CA, Labopin M, Sanz G, Michel G, Socie G, et al. KIR-ligand incompatibility in the graft-versus-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia. Leukemia 2009; 23:492-500.