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Stable mixed double donor chimerism

Absence of war doesn't necessarily mean peace

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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.

Key words: mixed chimerism, cord blood transplantation, HLA-C, allogeneic, SCT

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Double cord blood transplantation (DCBT), or when two-matched or partially-matched cord blood units are co-transplanted, is a clinical method that has successfully circumvented the problem of limited cell dose in a cord blood graft.[1-4] After what seems to be a short conflict between the transplanted cord blood units, one of the units usually prevails.[3,4] It has been suggested that the "winning" unit rejects the other unit, and thereby gains the space needed to reconstitute as the single new immune system of the recipient.[5] The pro-inflammatory milieu produced by the initial interaction may also induce collateral damage in the recipient's already conditioned immune system, which is possibly an explanation for the observed lower frequency of relapse in DCBT compared to single cord blood unit transplantations.[6]

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.[7] From an international perspective these patients are extremely rare,[4,8-10] 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.[11] 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.[12] As T cells after cord blood transplantation both reconstitute more slowly compared to adult stem cell sources[13] 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.[14]

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 and differentiate, but the alloreaction has not been strong enough for total rejection, 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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