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Double unit cord blood transplantation

Who wins—and why do we care?

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Double unit cord blood transplantation (DUCBT) has emerged as a successful strategy to improve engraftment and decrease transplant related mortality in adults and large children undergoing cord transplantation. In the vast majority of cases, one unit emerges as the sole source of long term hematopoiesis in the recipient following DUCBT. No factors have been identified that reliably predict which unit will emerge as the dominant unit, and limited studies have examined the mechanism underlying the observation. In a recent publication in *Blood*, we provide the first compelling data that effector CD8⁺ T cells play a critical role in the dominant unit actively rejecting the losing unit. Our findings provide an important first step in understanding the interactions following DUCBT, and provide insights that might be used to optimize graft versus leukemia effect and cord unit selection as well as better understand mechanisms of tolerance.

Key words: cord blood transplantation, double unit transplantation, chimerism, leukemia, stem cell transplantation

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The small number of total nucleated and CD34⁺ cells present in a single unit of umbilical cord blood contributed to a high incidence of graft failure and transplant related mortality in early studies of adult cord blood transplantation (CBT).[1,2] By combining two cord blood units to increase infused cell doses, the University of Minnesota pioneered a strategy that appears to have markedly improved engraftment rates and outcomes among adults,[3,4] and double unit cord blood transplantation (DUCBT) has become standard practice at many centers. In addition to improved engraftment rates, increasingly compelling clinical data suggests that DUCBT is associated with a reduced risk of disease relapse compared to other donor sources as well as an increased incidence of mild to moderate acute graft versus host disease (GVHD) as compared to single unit CBT.[3,5-7] DUCBT provides a unique opportunity to study in vivo interactions between two competent, albeit naïve, immune systems.

In the vast majority of cases, one unit emerges as the sole source of long term hematopoiesis in the recipient following DUCBT.[8] In our experience, after myeloablative conditioning regimens, the non engrafting unit is often not detectable in post transplant chimerism testing, even as early as 7 days post transplant. Following reduced intensity conditioning regimens, mixed chimerism is often detectable, though a dominant unit typically emerges by 28 days post transplantation. In a small number of patients, prolonged mixed chimerism, involving varying proportions of CD3⁺, CD56⁺ and CD33⁺ cells from each cord and the host, persists.[9] No factors have been identified that reliably predict which unit will emerge as the dominant unit, and limited studies have examined the mechanism underlying the observation.

We hypothesized that the emergence of a dominant unit was an immunologically mediated phenomenon, and in our recent Blood publication entitled "Single Unit Dominance Following Double Unit Umbilical Cord Blood Transplantation Coincides with a Specific CD8⁺ T Cell Response Against the Non-Engrafted Unit," we provide compelling evidence

that effector $CD8^+$ T cells play a critical role in the dominant unit actively rejecting the losing unit.[9] We investigated 14 patients who received a DUCBT. In 10 patients, dominant engraftment of a single donor unit emerged by day 28 after CBT. In 9 of these 10 patients, a significant subset of $CD8^+CD45RO^{+/-}CCR7^-$ T cells, present in peripheral blood mononuclear cells (PBMC) and derived from the engrafting cord blood unit, produced interferon-gamma (IFNγ) in response to the non-engrafting unit. No significant population of IFNγ secreting cells was detectable when post-transplantation PBMC were stimulated against cells from the engrafted unit ($p < 0.001$) or from a random human leukocyte antigen disparate third party ($p = 0.003$). Three patients maintained persistent mixed chimerism after CBT, and no significant IFNγ secreting cells were detected after similar stimulations in these patients ($p < 0.005$). We were unable to detect $CD4^+$ or NK cells reactive against the non-engrafted unit using IFNγ and CD107a secretion assays.

In their editorial accompanying our publication, reviewers noted that our work raises more questions than it provides answers, and we agree. Our data provides an important first step in understanding the interactions following DUCBT, but investigation is needed to better understand additional factors contributing to the emergence of the dominant unit and to exploit more reliably the potential benefits of DUCBT. Our data do not explain why mixed chimerism persisted in 3 patients with known HLA mismatches. A variety of other factors likely contribute to the "engraftment potential" of a cord blood unit, and further investigation to elucidate these factors is necessary. Recent work has suggested potential benefit, in the setting of single unit CBT, of transplantation with a unit sharing non inherited maternal antigens (NIMAs),[10] and perhaps NIMAs play a role in persistent mixed chimeras.

The apparent decreased risk of relapse following DUCBT is particularly intriguing. Based on our findings, we believe that decreased relapse may be related to the early post transplantation immunologic interactions between the two infused units and residual host cells. To investigate this further, we have successfully isolated from several patients the IFNγ producing $CD8^+$ T cells using IFNγ capture assays, have cloned and expanded individual T cells, have confirmed the cytotoxicity of these clones against cells derived from the non-engrafting unit using a chromium release assay, and have initiated experiments to determine the T cells' specific target antigens. Additional experiments are planned to examine whether T cells responding against the non-engrafting unit may share specificity against antigens on residual host hematopoietic elements. Even if T cells specific against shared antigens on both the non-engrafting unit and host cells cannot be identified, perhaps the inflammatory milieu created by the two cord units battling might contribute to the destruction of residual host cells. Several recent publications have suggested that infusion of haploidentical cells without intention of engraftment, perhaps mediated by NK alloreactivity, may cause a brief immunologic flair which results in decreased relapse rates.[11,12]

Finally, our data raise the possibility of designing a predictive assay which could be used to determine the dominant unit prior to transplantation. In general, following allogeneic stem cell transplantation, better matching is associated with less GVHD, better immune reconstitution, and less transplant related mortality and morbidity (TRM), but better matching also correlates with a higher incidence of relapse. Increasing evidence suggests that this observation is also true of CBT; better matched cord blood appears to correlate with better outcomes, at least in terms of TRM.[2,13] It is frequently difficult to find adequately sized well matched units. If the dominant unit could be predicted, a well matched smaller unit might be paired with a more poorly matched larger unit in order to facilitate engraftment. Though a better matched dominant unit might in theory lead to a higher relapse risk, perhaps the "battle" for dominance would enhance the graft versus leukemia effect while the ultimate dominance of a well matched unit might lead to decreased GVHD, more effective long term immune reconstitution, and lower TRM.

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