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Improving Clinical Outcomes Using Adoptively Transferred Immune Cells from Umbilical Cord Blood

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Abstract

Due to the necessary immunodepletion prior to cord blood transplantation as well as the immaturity of cord blood immune cells, recipients experience a high incidence of viral infection in addition to complications also observed after hematopoietic stem cell transplantation such as relapse and graft-versus-host disease. Here we describe current immunotherapeutic approaches to treat these complications, including the generation of antigen-specific T cells from cord blood, re-directing cord blood T cells using chimeric antigen receptors, and generating cord blood-derived NK cells and T regs.

Keywords

Immunotherapy; Immune Reconstitution

Umbilical Cord Blood Transplantation

The use of allogeneic stem cell products for the treatment of hematological malignancies and genetic disorders has increased the likelihood of long term survival for a significant number of patients.[1], [2] Unfortunately, bone marrow and stem cell transplants require rigorous protocols and matching requirements to reduce the incidence of graft-versus-host disease.[3] Due to these stringent requirements, minorities often lack suitable stem cell donors.[3] However, the use of umbilical cord blood (CB) as an alternative source of hematopoietic stem cells has become increasingly popular. According to data from the Eurocord Registry, the number of unrelated cord blood transplants (CBT) has increased ~800% between 1997 and 2007.[4] Cord blood is therefore an attractive source of hematopoietic stem cells – particularly for minorities – for several reasons: first, over 400,000 frozen cord blood units are readily available at over 100 cord blood banks throughout the world.[5], [6], [7] Second, recipients of CB grafts tolerate greater human leukocyte antigen (HLA) mismatches when compared to peripheral blood stem cell or bone marrow graft sources.[8], [9], [10], [11], [12], [12], [13], [14], [15] Lastly, despite the greater tolerance to HLA disparity (and less Graft-versus-host disease, GvHD), cord blood grafts have been reported to exhibit graft-vs-leukemia (GvL) effects, keeping the rate of relapse after cord blood transplantation (CBT) equal to or less than after hematopoietic stem cell transplantation.[16], [17], [18], [19]

Notwithstanding the many advantages of utilizing CB in transplantation, CB as a graft source has some unique properties compared to peripheral blood stem cell or bone marrow graft sources. Immune cells from cord blood are relatively antigen inexperienced, or naïve. [20] While our understanding of the relationship between the naïveté of CB T and NK cells and their effect on GvL and GvHD is still lacking, we do know that upon mitogenic stimulation, CB-derived immune cells have reduced cytolytic ability and cytokine secretion. Moreover, the antigen presenting cells are immature and express less adhesion molecules when compared to peripheral blood (PB) derived immune cells.[21], [22], [23], [24] Hence, the purpose of this review is to describe how to exploit the aforementioned advantages of CB while overcoming the limitations.

Immune Reconstitution after CBT

Immunodepletion is an undesired yet necessary step in most myeloablative regimens. In CBT, the subsequent immune reconstitution is significantly delayed after transplant. Although NK cells and other cells of the innate immune system begin to recover within weeks after CBT, T and B lymphocytes do not begin to reconstitute until later, beginning with B cells 3–6 months after transplant and T cells between 9–12 months after transplant. [25] While total white blood cell counts normalize around 1 year post CBT, the ratio of CD4 to CD8 T cells does not normalize until approximately 2 years after CBT.[26], [27] Furthermore, the delayed immune reconstitution and naïveté of cord blood lymphocytes has been attributed to the increased susceptibility of CB recipients to infection. One report attributes greater than 50% of deaths after CBT to infection.[12] Another study performed at Duke University evaluated 330 pediatric recipients who received CBT and observed a TRM rate of 33%, with 58% of deaths due to opportunistic infections associated with T cell deficiency.[28], [27]

Expanding CB T cells for Donor Lymphocyte Infusion

The use of unmanipulated donor T cells to prevent relapse as well as viral infections has shown promise after HSCT.[1] Despite the beneficial GvL effect from donor lymphocyte infusion (DLI), the administration of unmanipulated lymphocytes can result in an increased risk of GvHD. In the setting of CBT, it is not feasible to isolate sufficient numbers of unmanipulated donor lymphocytes from the product for DLI. Furthermore, it is not possible to go back to the donor source. For this reason, groups have investigated the possibility of expanding CB-derived T cells ex vivo for infusion post CBT. The feasibility of this approach has been demonstrated by Okas et al, where beads against CD3 and C28 (along with IL-2) were used to expand CB T cells. Resulting T cells showed a mean-fold expansion of 148, starting with as few as 1.5×10^6 CD3+ cells.[29] More recently, Davis et al have suggested that the use of IL-7 in CB T cell cultures increases cell survival and T cell function after expansion.[30] Although still being tested in vivo (Table 1), the use of ex vivo expanded CB donor T cells could help rapidly restore T cell immune reconstitution after CBT.

Generating and Utilizing Antigen-specific Cytotoxic T Lymphocytes from Cord Blood

Due to the antigen-inexperienced nature of cord blood, there are a higher percentage of naïve T cells in CB than in peripheral blood (PB).[20] Memory T cells are critical in non-T cell depleted grafts because the virus-specific memory T cells present in the grafts confer protection against viral infections and reactivation. For this reason, CMV-reactivation is highest when the transplant recipient is CMV-seropositive and the transplant donor is CMV-seronegative, as is often the case in CBT.[31]

Memory T cells are also a valuable resource when expanding virus-specific T cells ex vivo from seropositive donors as these T cells can be expanded by simply culturing virus-specific memory T cells with antigen-presenting cells loaded with the antigen of interest. When transferred to transplant recipients after transplant, these cells have been protective against CMV, EBV, and adenovirus without severe adverse events.[32], [33] The challenge, however, has been the ex vivo generation of antigen-specific T cells from antigen-inexperienced sources of T cells such as cord blood. Instead of expanding the pre-existing memory T cell population, naïve T cells need to be primed in vitro to respond to the antigen of interest. The inherent problems with attempts to generate antigen-specific T cells from cord blood include the naivete of the T cells, the need to mimic a more natural priming environment, the reported deficiency in NFAT signaling in CB T cells, and their innate Th2 bias.[34], [35], [36]

Because of these challenges, only a few reports document the generation of antigen-specific T cells from cord blood. Sun et al first reported the ability to generate EBV-specific CD4+ T cells using EBV-transformed B-cells, or lymphoblastoid cell lines (LCL).[37] Parks et al then reported the ability to generate CMV-pp65-specific T cells from cord blood by using CMV-immune complex-loaded DCs, CMV lysate, and IL-12 and IL-7.[38] Shortly after, our group reported the ability to generate CMV, EBV, and adenovirus-specific CTL from the 20% fraction of a cord blood unit by using dendritic cells transduced with an Ad5/f35-CMV-pp65 vector as well as the cytokines IL-7, IL-12, and IL-15.(Figure 1) Responding T cells were shown to be derived from the naïve T cell population and responded to unconventional and novel CMV-pp65 epitopes.[39] A clinical trial using CB-derived multi-virus specific T cells for the prevention and treatment of viral infection after CBT is open and has started to accrue patients. (Table 1; Clinical Trial #: NCT01017705).

The use of cord blood-derived antigen-specific T cells is however not limited to virus-specific T cells as highlighted in a study by Merindol et al recently expanded MART-1-specific T cells from umbilical cord blood by culturing CB mononuclear cells (CBMC) with HLA-A2+ LCLs pulsed with the HLA-A2-restricted modified Melin-A A27L peptide. Importantly, the resulting CTL lines exhibited less alloreactivity than CTL generated using the same method derived from the PB of adult donors.[40] Moreover, while the biology and understanding of cord blood T cells is still not well defined, the indispensable role of gamma-chain cytokines such as IL-7 and IL-15 have become apparent as most methods published to date using GMP-approved protocols for the generation of antigen-specific T cells from umbilical cord blood utilize either IL-7, IL-15 or both.[39], [38], [40]

Redirecting specificity of CB-derived T cells to Leukemia antigens

Targeting malignancies using exogenous receptors (often antibodies) expressed on peripheral blood ex vivo-expanded T cells (known as chimeric antigen receptors, or CARs) is an evolving therapy for both hematologic malignancies and solid tumors.[41], [42] In recent years this approach has been translated to the cord blood setting by expanding CB-derived T cells that are genetically modified to express CD19 or CD20 CARs.[43], [44] Typically CARs utilize monoclonal antibodies to direct specificity to the antigen of interest. However, T cells redirected with a CAR lack signaling through a native TCR and sufficient costimulation, which has resulted in their poor persistence in vivo.[45], [46], [47] Recent advances have tethered costimulatory endodomains to the transmembrane and/or exodomain of the CAR with the intention of inducing IL-2 production and upregulation of pro-survival genes. Other signaling molecules have also been tested in second generation CAR – transduced T cells derived from both peripheral blood and umbilical cord blood, including 41BB, CD28, and OX40 with promising results in vitro but are largely untested persistence in vivo.[48], [49], [50], [51], [52], [53], [54], [55], [56]

To generate CB-derived CAR-expressing T cells, T cells can be non-specifically expanded by mitogenic stimulation and subsequently transduced with a retrovirus, DNA plasmid, or transposon system.[57] Though adequate T cell numbers can be achieved using this methodology, the T cells are non-specific and only retain the specificity of the CAR.

Recently, Micklethwaite et al expanded the concept by transducing antigen-specific T cells derived from umbilical cord blood with a (CAR) against the B cell malignancy marker CD19. These cells coexpress endogenous TCRs for three of the most common viruses that affect patients after cord blood transplant (CMV, EBV, and adenovirus) as well as the CAR CD19.[58] The rationale of this approach is that while the CB-derived T cells still express the CD19+ CAR, the re-directed T cells exploit the native virus-specificity of the endogenous TCR and costimulatory molecules, which receive constant stimulation via latent viral antigens as was described in the neuroblastoma setting.[59] In this way the T cells should persist longer while retaining anti-tumor efficacy, a requirement for protecting against relapse after transplant.

Cord Blood-derived NK Cells

Natural Killer cells are innate immune cells that recognize “non-self” by the absence of Class I molecules and inhibitory receptors. Although the proportion of NK cells in cord blood is similar to that of adult peripheral blood, the NK cells found in CB are immature both in phenotype and function.[60], [61], [62] NK cells from CB have been reported to express less of the activation receptor NKG2C and more of the inhibitory receptors NKG2A and KIRs.[63] CB NK cells also express less granzyme and are hence are less cytotoxic than PB NK cells.[64]

Ruggeri et al have demonstrated that PB-derived NK cell alloreactivity is capable of preventing relapse of acute myeloid leukemia (AML) after HLA-mismatched haploidentical stem cell transplant.[65], [66] Importantly, these cells did not correlate with a concomitant increase in acute GvHD. NK cells have also been used as a successful immunotherapeutic tool where haploidentical NK cells were infused to poor-prognosis patients with AML who did not receive a transplant.[67] What is encouraging is that despite evidence of graft-vs-leukemia effects, there was not an increase in GvHD. Still, this phenomenon needs further investigation as the degree of GvHD and GvL varies between studies and how the graft (and likely NK cells) prevents relapse remains to be elucidated.

Because of the promise of allogeneic NK cell therapy, several groups have developed methods to isolate NK cells from peripheral blood of healthy donors, primarily by leukaphoresis and subsequent isolation by magnetic beads.[68], [69] However, one significant limitation when using cord blood-derived products is the finite volume of blood and cells from cord blood. This limitation has led to the development of methods to expand NK cell numbers in vitro. One method developed by Spanholtz et al isolates CD34+ progenitor cells seeded in H3000 medium or Stemline II medium containing a cytokine cocktail (that includes IL-7). Using this method NK cells expand by 3 logs from frozen cord blood.[70] Another, simpler approach was recently described where CD3⁻/CD56⁺ cells were magnetically selected and then expanded in T-25 flasks in media containing human serum and 500 U/mL IL-2. After expansion, the cord blood NK cells had cytolytic function in vitro against AML blast targets. Further, when these expanded CB NK cells were transferred to a NOD-SCID-IL2R^γnull mouse model of human AML, the NK cells exhibited in vivo anti-leukemic activity, suggesting the potential of using donor-derived CB NK cells to prevent relapse.[71]

Recently, a phase II clinical trial used peripheral blood derived NK cells (CD3-depleted cells) for patients with AML who had detectable disease after HSCT. Of 15 patients, two

reached the primary end point of 6-month disease-free survival (Clinical Trial # NCT00354172). Whether ex-vivo expanded CB NK cells will be more efficacious in this setting remains to be tested.

Exploiting CB-derived Regulatory T cells to Prevent GvHD

Regulatory T cells (Tregs) are a lineage of T cells possessing the ability to inhibit other T cell responses. Naturally occurring Tregs are CD4+, CD25+, express the forkhead box protein 3 (FoxP3), and represent 1–5% of circulating CD4+ T cells.[72] In the transplant setting, the adoptive transfer of donor Tregs has been explored as a strategy to decrease the severity of acute GvHD and as treatment for chronic GvHD.[73], [74], [75]

Unfortunately, the translation of successful pre-clinical data into the clinic has been lacking due to the difficulty in isolating high numbers of sufficiently pure PB derived Tregs. Expanding PB Tregs to sufficient numbers is also a challenge. After isolating CD4+ CD25+ T cells, there are still activated T cells in the culture necessitating the addition of rapamycin to the culture, ultimately limiting the expansion of the Tregs.

Despite the successful utilization of Tregs in murine models, the translation of this approach to the clinic has been slower as a result of the difficulties outlined above. While this problem is exaggerated in cord blood due to the finite volume of blood available, CB Tregs have a more distinguishable CD4+ CD25+ population and after isolation can be subsequently expanded using a variety of Treg expansion protocols.[76], [77] Tregs from CB expand 4–10 times better than Tregs from PB, allowing a 200–1000 fold expansion in less than three weeks.[76], [77] This expansion is achieved by culturing isolated Tregs with anti-CD3 and anti-CD28 microbeads that cross link the T cell receptor and CD28 and supplementing the culture with exogenous IL-2. Resulting Tregs can suppress effector T cells at ratios as low as 1 CB Treg to 3 effector T cells. Moreover, these CB-derived Tregs have been used clinically by the Minnesota group who have transferred HLA partially-matched Tregs to 19 transplant recipients. Results from this study appear encouraging as evidenced by a delayed time to GvHD (compared to historical controls), and an increase in circulating Tregs.[78]

Conclusions

Despite the many advantages of utilizing CB as a source of allogeneic stem cells, utilizing CB immune cells after transplant still lags far behind advances in the utilization of peripheral blood immune cells. The impaired activation, cytokine secretion, and proliferation of most CB immune cells -in addition to their overall immaturity—forces investigators to develop novel immunotherapeutic strategies. Such approaches include previously published methodologies including gamma chain cytokines and genetic modifications, as well as cutting-edge technologies that target and manipulate CD34+ progenitor cells as well as genetic modifications. More importantly, the majority of the strategies outlined in this review still need to be translated to the clinical setting to enable researchers to evaluate and optimize the successes and failures of these approaches. In conclusion, these CB-derived immune based therapies will serve to address critical areas of CB transplantation (Figure 2) that have the potential to improve the outcomes of CBT in patients. Ultimately, these efforts will allow transplant physicians to offer patients a comprehensive plan of CBT by restoring antiviral immunity and reducing GVHD while preventing relapse.

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Figure 1. Methodology used to generate CB-derived CMV, EBV and adenovirus-specific CTL MNC are isolated from the 20% fraction of the cord blood unit, from which DCs and LCL are generated. Non-adherent cells (remaining after the adherence of monocytes on day 0) are frozen until initiation. On day 5 DCs are transduced with the Ad5f35CMVpp65 vector. On day 7, T cells are cocultured with irradiated (30Gy) DCs at an E:T ratio of 20:1, with cytokines IL-7, IL-12, and IL-15. After ~10 days the CTL are frozen until the LCL are ready. Once sufficient numbers of LCL are available, they are transduced with the same Ad5f35CMVpp65 vector, and after 2 days are irradiated (40Gy) and used to stimulate the thawed CTL at an E:T ratio of 4:1 in the presence of IL-15. Transduced LCL are then used to stimulate and expand virus-specific CTL with IL-2.

Figure 2. Immunotherapeutic strategies to enhance Cord Blood Transplantation

Emerging immunotherapeutic strategies from cord blood in pre-clinical or clinical development will potentially be used to treat relapse (NK cells, re-directed T cells, DLI), virus infection (virus-specific T cells and re-directed T cells), and GvHD (Tregs).

Table 1

Clinical trials utilizing immune cells from umbilical cord blood, as listed on www.clinicaltrials.gov

Cell Type	Treating:	Clinical Trial #	Study Start Date	Phase	Institution
Donor Lymphocyte Infusion	Relapse	NCT00972101	2009	I	M.D. Anderson Cancer Center
Donor Lymphocyte Infusion	Relapse	NCT00281879	2006	I	Knight Cancer Institute at Oregon Health and Science University
Virus-specific T cells	Virus infection	NCT01017705	2009	I	Baylor College of Medicine
NK cells	Relapse	NCT00354172	2006	II	Masonic Cancer Center at University of Minnesota
NK cells	Relapse	NCT00871689	2007	II	Masonic Cancer Center at University of Minnesota
Tregs	GvHD	NCT00602693	2007	II	Masonic Cancer Center at University of Minnesota
Tregs	GvHD	NCT00376519	2007	I	Masonic Cancer Center at University of Minnesota