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Adoptive Immunotherapy using Regulatory T cells and Virus-specific T cells Derived from Cord Blood

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Abstract

Cord blood transplantation, an alternative to traditional stem cell transplants (bone marrow or peripheral blood stem cell transplantation), is an attractive option for patients lacking suitable stem cell transplant donors. Cord blood units have also proven to be a valuable donor source for the development of cellular therapeutics. Virus-specific T cells and regulatory T cells are two cord blood derived products that have shown promise in early phase clinical trials to prevent and/or treat viral infections and graft-versus-host disease (GvHD), respectively. Here we describe how current strategies utilizing cord blood-derived regulatory T cells and virus-specific T cells have been developed to improve outcomes for cord blood transplant recipients.

Keywords

Regulatory T cells (Treg); cord blood; transplant; GvHD; T cell; immunotherapy; cell therapy; antiviral; virus

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Introduction

Umbilical Cord blood (UCB) has been shown to be a valuable alternative donor graft source for allogeneic hematopoietic stem cell transplantation (HSCT). Worldwide, there are about 600,000 CB units stored for clinic use. While the main application of UCB is as an allogeneic stem cell source, these units may be also used as a donor source of cells (1)for the development of novel cell therapeutics. The unique immunological properties of UCB present both challenges and opportunities for these applications. The naiveté of the UCB immune system necessitates novel manipulations for the development of antigen specific T cells. In contrast, the unique properties linked to materno-fetal tolerance make UCB an excellent source of regulatory T cells. In this manuscript we review the utilization of UCB-derived cells as a source of both multi-virus-specific T cells (mTC), for the treatment and prevention of viral infections, and natural regulatory T cells (Treg), for the suppression and treatment of GVHD.

Adoptive Transfer of Regulatory T cells (nTregs)

Regulatory T cells (Treg) help modulate responses mediated by effector T cells to avoid an autoimmune response in vivo. (2) Individuals that are born with a functional deficiency of naturally occuring Tregs (nTreg) develop severe auto-immunity syndrome known as IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome). (3) Tregs are CD4+ CD25hi T cells that express the FoxP3 transcription factor and more recently, have also be shown to express low levels of CD127, the interleukin (IL)-7 α-chain receptor. (4, 5) Notably, Tregs depend on IL-2 secreted by other T cells for survival and proliferation. (2) More recently, the results from several groups have improved our understanding of Treg biology as well as the potential clinical application of these cells not only to reduce the risk of acute graft versus host disease (GVHD) after allogeneic transplantation, (6–12) but also to suppress graft rejection after solid organ transplantation (13) and the treatment of auto immune diseases. (14)

The clinical application of Tregs requires approaches that have typically utilized CD25 positive selection from peripheral blood or umbilical cord blood (UCB) donor sources as follows: 1) Treg infusion with or without the administration of IL-2 to promote Treg expansion in vivo, 2) ex vivo expansion/activation of Tregs prior to infusion, and 3) ex vivo expansion/induction of the Treg (iTreg) phenotype followed by infusion. (15) Currently, in clinicaltrials.gov there are over 10 clinical trials evaluating the adoptive transfer of Tregs for the treatment or prevention of GVHD after HSCT or graft rejection after solid organ transplantation or for the treatment of autoimmune diseases (e.g. type 1 diabetes and Crohn’s disease). Among the numerous studies that have evaluated Tregs clinically, one study using UCB-derived Tregs has been reported with promising results. (16, 17)

The choice to develop an UCB-derived Treg strategy was based on pre-clinical studies that demonstrated a distinct population of CD4+CD25hi T cells in UCB, responsible for maternal-fetal tolerance. (18) This population could be easily delineated and after expansion/activation in culture these cells were reproducibly suppressive. (19) In contrast to peripheral blood, only one selection step based on CD25 expression is required to expand

Tregs from UCB and the expansion culture does not require sirolimus to prevent T effector outgrowth. After CD25 selection, the resultant cell population is ~60% CD4+CD25+FoxP3+CD127-. The expansion methodology has undergone an evolution over time. (16) Patients undergoing a double UCB transplant for hematological malignancies received partially HLA matched UCB derived Tregs obtained from a third unit (partially matched with the patient and hematopoietic stem cell graft). In the first 23 patients, CD25+ T cells were cultured in the presence of beads coated with anti-CD3/anti-CD28 and supplemental IL-2. After passing lot release UCB-derived Tregs were infused the day after UCB transplantation in order to monitor for infusion-related side effects. Important observations from this initial study were the favorable profile of ex vivo expanded UCB-derived Tregs with no infusion related severe adverse events. There were no deleterious effects clinically, and there was a reduction in the risk for the development of grade 2–4 acute GVHD (Figure 1). After a minimum follow up of 2 years, no adverse effects on treatment failure and mortality were identified in the Treg recipients compared to historical controls. (20) There was a suggestion of an increased risk of viral reactivation specifically within the first 30 days, but the historical comparison was limited as viral testing was not available during most of the time the historical controls were treated and could represent an observation bias. Nevertheless, cautious monitoring for viral reactivation in such Treg adoptive transfer studies is warranted.

In the initial clinical trial, albeit in small numbers, Tregs were detectable in the peripheral blood up to 14 days after infusion (21). This detection was based on flow cytometry for the expression of HLA antigens that were different between the Treg donor unit and the patient and two donor UCB units. For example, the Treg units were HLA-A2 positive, whereas the patient and the two donor units were HLA-A2 negative. The length of persistence in the peripheral blood was similar to what was observed in the murine models of GVHD. As the early contact between donor cells and recipient antigens are critical for the development of GVHD, the presence of Tregs early post infusion of the graft is desirable. However, long term persistence on Tregs may not be required to suppress GVHD and could potentially lead to an increased risk of relapse as seen after in vivo or ex vivo T-cell depletion. In mice, the presence of Tregs in lymphoid tissues has been shown (10). While it would be of great interest to document whether or not adoptively transferred Tregs persist long term in lymphoid tissues, we do not yet have a practical and medically appropriate way to do it as it would take a lymph node biopsy.

However, higher Treg doses are desired in order to achieve the target T effector to Treg ratio of 1:1. A modified methodology included expansion using K562-based artificial antigen presenting cells (aAPC) that express the high affinity receptor for the Fc portion, loaded with anti-CD3 antibody, and CD86, the natural ligand of CD28/CTL-4 (KT64/86). The use of these aAPC resulted in a greater expansion of Tregs in vitro compared to the bead-based methodology. (22, 23) In addition, higher doses of Treg were possible with a single restimulation with the KT64/86 aAPCs. This methodological advance ensures that Treg cell doses of 100×10^6/kg can be obtained. A phase I dose escalation trial is currently underway to test the safety and potential efficacy of high dose UCB-derived Treg expanded using aAPC with promising early results (NCT00602693).

Other promising adoptive Treg strategies are under investigation by several groups worldwide generally using adult peripheral blood as the donor source (17). However, another promising UCB strategy is under development at the MD Anderson Cancer Center applying fucosylation to ex vivo expanded UCB-derived Tregs in order to promote Treg engraftment to enhance the anti-GVHD effect (personal communication S. Parmar and E.J. Shpall). (24, 25) Their clinical trial is expected to open in 2015.

As therapeutic strategies utilizing Tregs are translated to the clinic, the importance of HLA-matching (especially when using third party Tregs) and antigen specificity will need to be considered in the study design. Murine models of solid organ and skin allografts have shown better graft survival and less rejection when antigen-specific Tregs are administered (26, 27). In this setting, Treg recognition of antigen present on recipient cells resulted in more efficient suppression of the alloreactive response. (1) However, in a murine model of GVHD, both donor and recipient derived Tregs were able to suppress GVHD.(10) Current studies have used partially HLA-matched UCB-derived Treg from a third party donor. (21) In addition, a clinical trial for patients after haploidentical donor transplant used Tregs derived from the same donor, thus HLA identical to the hematopoietic cell graft (17). While there is a theoretical concern that HLA disparity between donor and/or recipient Tregs could result in rejection of the adoptively transferred Tregs, the clinical data utilizing partially HLA-matched UCB-derived Tregs does not suggest this occurs in vivo. Furthermore, the immunological naiveté of cord blood effector cells, the role of neonatal Tregs in feto-maternal tolerance, and the ease of access to cord blood units make cord blood an attractive donor source for Treg expansion and adoptive therapy. However, it still remains to be determined whether this “off the shelf” third party Treg approach will effectively prevent and/or treat GVHD in patients after HSCT.

In summary, the adoptive transfer of UCB-derived Tregs for the suppression of GVHD is promising. The ability to produce large numbers of Tregs from a single UCB unit by either bead or aAPC stimulation/expansion has the potential for the development of a cryopreserved Treg “off the shelf” product that would be readily available for clinical use. Ongoing and planned studies will further define the clinical efficacy of this cell therapy for the suppression of acute GVHD as well as other clinical contexts beyond GVHD.

Adoptive Transfer of Virus-specific T cells (mTC)

In addition to GvHD and relapse, one of the biggest risks of morbidity and mortality after stem cell transplant are viral infections, most notably from Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and adenovirus. (28) The risk for viral infections varies and is dependent upon the donor source, conditioning regimen and the degree of T cell depletion. More than 1/3 of deaths after alternative donor stem cell transplant are attributed to viral infection. (28) Antiviral pharmacotherapy drugs do exist but are associated with unacceptable side effect profiles and are not always effective. (29, 30) The adoptive transfer of ex vivo-expanded virus-specific T cells is an appealing alternative to patients at high risk for viral infection, or those who cannot tolerate or have failed conventional pharmacotherapies. (31–34)

The clinical use of virus-specific T cells after stem cell transplant was first reported in 1992 by Riddell, Greenberg, and colleagues. In this study, CMV-infected fibroblasts were used to expand donor-derived CMV-specific T cells prior to infusion. Infused T cells were safe, did not cause GvHD, and provided reconstitution of CD8+ CMV-specific T cells in patients after HSCT. (32) Over the last twenty years, advances in technology and manufacturing have led to less complex T cell expansion procedures, reduced the culture time, and eliminated or limited the use of live virus during production. (35) Notably, in some cases the virus specific T cells were immediately available as an off-the-shelf product. (36–39) Listed in Table 1 are current methods of T cell expansion or selection. However, the limitation of all these methodologies is that they all require prior viral exposure. Additional methodologies have been used to generate virus-specific T cells from naïve donors, especially for EBV, but these methodologies are more extensive than those listed in Table 1 and often involve multiple selections and manipulations. (40–42)

Significant advantages as well as challenges exist when trying to utilize UCB as a source for adoptive cellular immunotherapy. A major advantage is that UCB is a readily available “off the shelf” donor source. However, since the units are already collected and cryopreserved, any manipulation of the cells will likely need to be done upon thawing – saving aliquots of the cells for later use is not an option unless the bags are partitioned into 20% and 80% fractions; even still, using both fractions of the unit is only an option in the third party setting because in CBT, the 80% fraction would need to be transferred to the recipient as part of their cord blood transplant. For this reason, UCB donor-derived donor lymphocyte infusion is not an option after cord blood transplantation (CBT) unless it is ex vivo expanded at the time of thaw. (43, 44) Another challenge is the naiveté of the cord blood immune cells. (45) The majority of UCB T cells express naïve T cell markers such as CCR7, CD62L, and CD45RA. Further, UCB denditric cells (DC) were reported to be less potent, (46, 47) and to secrete less IL-12, which is critical cytokine for T cell priming. It has also been suggested that UCB T cells are less cytotoxic than peripheral blood derived T cells. (48)

For these reasons, the majority of the GMP-compliant expansion and selection methods for virus-specific T cells from virus-experienced donors are not an option when virus naïve donors (e.g. UCB) are used– at least not yet. The rapid expansion of T cells using mononuclear cells is not currently an option because the naïve T cells with T cell receptors recognizing viral epitopes are at a lower frequency than memory virus-specific T cells in the peripheral blood. In fact, antigen-specific T cells from the naïve population require optimized priming conditions, such as the use of professional antigen presenting cells like dendritic cells, as well as cytokines that favor the priming of naïve T cells such as IL-7. (49, 50)

The first report of a virus-specific T cell line derived from UCB came from Sun et al in 1999 (50) where EBV-specific T cells were expanded from UCB using autologous EBV-transformed lymphoblastoid cell lines (LCL) in the presence of IL-2. In 2006, Park et al reported the in vitro priming of CMV-specific cord blood T cells using UCB dendritic cells pulsed with CMV lysate as stimulators in the presence of IL-7 and IL-12. In this study, after 4 weeks ex vivo expansion, the majority of the T cell product was comprised of CD45RO+ memory T cells that secreted IFN-g, IL-2, and TNF-a. However, expansion was relatively

limited (2–5 fold) which was problematic for clinical translation. (49) This seminal report led us to later report the GMP-applicable expansion of UCB-derived multi-virus T cells (mTC) recognizing CMV, EBV, and adenovirus to the numbers required for clinical use. (51) Moreover, by utilizing the Grex culture device, these mTC could be expanded to clinically relevant numbers ($>6\times10^7$) using only the 20% fraction of thawed UCB units providing a donor-specific mTC product for patients after CBT. (51–53) mTC required the priming of naïve T cells with dendritic cells in addition to IL-7 and IL-12, (49) as well as the addition of IL-15 to prevent activation-induced cell death (54).

To date, 9 patients have been treated at the Center for Cell and Gene Therapy (Baylor College of Medicine and Texas Children's Hospital) with UCB-derived mTC. All infusions were well tolerated and not associated with the development of GvHD. (55) Importantly, reserving the 20% fraction of the UCB unit for mTC manufacture did not result in delayed engraftment, with a median time-to-neutrophil engraftment of 21 days. Clinically, three patients had viral reactivation or infection: 1 patient had CMV reactivation and adenovirus infection, and 2 patients had EBV reactivation. All three patients resolved their viral infections and we were able to detect the adoptively transferred virus-specific T cells in the peripheral blood by interferon gamma ELISPOT assay and/or deep T cell receptor sequencing. (55)

To further extend the application of CB-derived mTC as a therapeutic, these cells were transduced with a retrovirus vector expressing a chimeric antigen receptor (CAR) targeting CD19, present on many B cell malignancies including ALL and CLL. The resultant T cell product had antiviral specificity through the endogenous T cell receptor and anti-leukemic specificity through the CD19-CAR. (56) This approach is now being used clinically in the peripheral blood setting to prevent and treat virus infection and leukemia relapse after HSCT, and a similar study for patients after CBT is planned. (57)

In summary, the adoptive transfer of UCB-derived mTC to prevent and treat viral infection after CBT is feasible and early phase studies suggest that the approach has an excellent safety profile. A subsequent study is currently evaluating the administration of UCB-derived mTC generated without gene-modified autologous APC for expansion (NCT01923766), as well as strategies to decrease the mTC manufacturing time and to extend the virus panel beyond CMV, EBV, and adenovirus.

Conclusions

Adoptive immunotherapy from cord blood cells has gained momentum in recent years due to new technological advances as well as the increased use of cord blood as a graft source. While UCB has some disadvantages, the fact that this donor source is immediately available, well characterized, and contains mostly naïve lymphocytes also makes it an ideal candidate for immunotherapy. In the case of Tregs, the high expression of CD25 makes UCB an ideal starting cell population, and in the case of virus-specific T cells, the ability to manufacture antiviral therapies personalized to the recipients of cord blood transplants, including minorities, is extremely beneficial. There is continued interest in UCB as a unique donor source and with the results from current as well as planned clinical studies using UCB-

derived Tregs and mTC (Table 2) it will be possible to better define the clinical efficacy profile and application of these novel cell therapies even beyond the HSCT/CBT setting.

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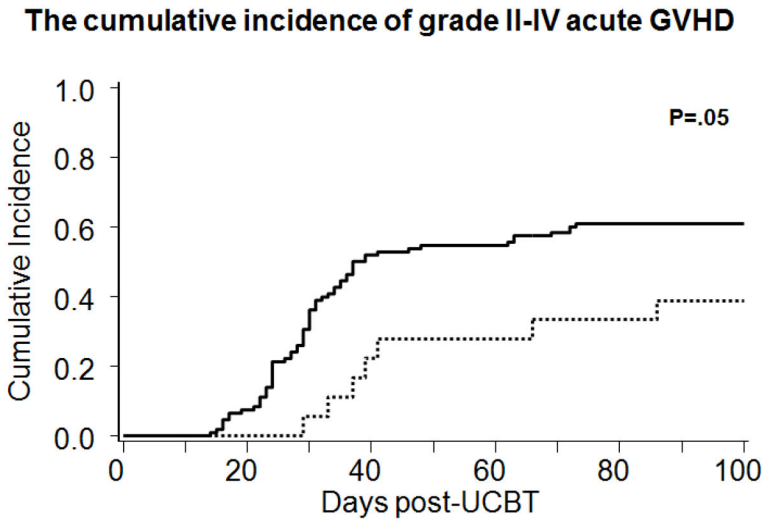

Figure 1.

the cumulative incidence of garde II-IV acute GVHD by 100 for patients who received ex vivo expanded Tregs (---)and historical controls (—). (Adapted from Brunstein et al, 2011;117(3):1061–70).

Table 1

Current selection or expansion strategies for virus-specific T cells

Method	Culture period (including release testing)	Intermediaries	Reference(s)
Rapid multi-virus T cells	13–20 days	None	Papadopoulou et al 2014 (29); Gerdemann et al 2013 (58); Gerdemann et al 2012 (35)
Peptide-specific expansion	21–30 days	Dendritic cells	Micklethwaite et al 2007 (59)
Gamma-selection	1–2 days	None	Peggs et al 2011 (34)
Tetramer selection	1–2 days	none	Cobbold et al. 2005 (60); Mackinnon et al 2008 (61); Luo et al 2010 (62)
Multi-virus T cells with engineered adenoviral vector	1–3 months (including LCL generation)	EBV-LCL, monocytes or dendritic cells	Leen et al 2006 (31), Micklethwaite et al 2008 (33); Hanley et al 2010 (63)

Table 2

UCB Adoptive T cell and Treg therapies in clinical trial

Cord Blood Therapy	Method	Intermediaries	Cord Blood Source	Clinical Trial Number
Tregs (15,19,20,21)	CD25 selection, CD3/CD28 bead and KT64/86 stimulation	Genetically modified K562	Third party	NCT00376519
Tregs (20,21)	CD25 selection, KT64/86 stimulation	Genetically modified K562	Third party	NCT02118311
mTC (55)	T cell stimulation with Ad5f35pp65-transduced APCs	DCs, EBV-LCL	Donor-derived (20% fraction)	NCT00880789
mTC	T cell stimulation with overlapping peptide-pulsed APCs	DCs, EBV-LCL	Donor-derived (20% fraction)	NCT01923766
DLI (64)	CD3 selection, CD3/CD28 bead stimulation	None	5% of donor UCB	Not available
DLI (65)	CD3 selection, CD3/CD28 bead stimlulation	None	UCB Donor-derived	NCT01630564