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Adoptive immunotherapy with the use of 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, respectively. We describe how current strategies that use cord blood–derived regulatory T cells and virus-specific T cells have been developed to improve outcomes for cord blood transplant recipients.

Key Words: *antiviral, cell therapy, cord blood, graft-versus-host disease, immunotherapy, regulatory T cells (Treg), transplant*

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. Although 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 report, 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 (Tregs), for the suppression and treatment of graft-versus-host disease (GVHD).

Adoptive transfer of Tregs

Tregs help modulate responses mediated by effector T cells to avoid an autoimmune response *in vivo* [2]. Individuals who are born with a functional deficiency of naturally occurring Tregs develop severe auto-immunity syndrome known as immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) [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 GVHD after allogeneic transplantation [6–12] but also to suppress graft rejection after solid organ transplantation [13] and the treatment of autoimmune diseases [14].

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The clinical application of Tregs requires approaches that have typically utilized CD25 positive selection from peripheral blood or UCB donor sources as follows: (i) Treg infusion with or without the administration of IL-2 to promote Treg expansion *in vivo*, (ii) *ex vivo* expansion/activation of Tregs before infusion and (iii) *ex vivo* expansion/induction of the Treg phenotype followed by infusion [15]. Currently, in clinicaltrials.gov there are more than 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 (eg, type 1 diabetes and Crohn's disease). Among the numerous studies that have evaluated Tregs clinically, one study that used UCB-derived Tregs has reported promising results [16,17].

The choice to develop a 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 human leukocyte antigen (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 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 grades 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 with historic control subjects [20]. There was a suggestion of an increased risk of viral reactivation specifically within the first 30 days, but the historic comparison was limited because viral testing was not available during most of the time the historic control subjects were treated and could represent an

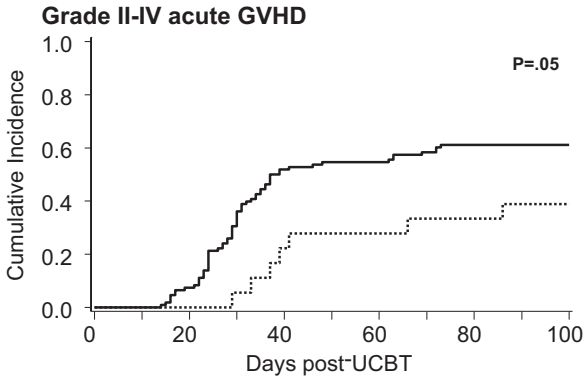

Figure 1. Cumulative incidence of grade II-IV acute GVHD by day 100 for patients who received *ex vivo*–expanded Tregs (···) and historic control subjects (—). Adapted from Brunstein *et al.* [16].

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 [16]. 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. Because the early contact between donor cells and recipient antigens are critical for the development of GVHD, the presence of Tregs early after 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]. Although 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 because it would require a lymph node biopsy.

However, higher Treg doses are desired to achieve the target T-effector–to–Treg ratio of 1:1. A modified methodology included expansion with the use of K562-based artificial antigen-presenting cells (aAPCs) 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 aAPCs resulted in a greater expansion of Tregs *in vitro* compared with the bead-based methodology [21,22]. 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