Autologous Umbilical Cord Blood Transfusion in Young Children With Type 1 Diabetes Fails to Preserve C-Peptide

Michael J. Haller, md[1]
Clive H. Wasserfall, ms[2]
Maigan A. Hulme, bs[2]
Miriam Cintron, bs[1]
Todd M. Brusko, phd[2]
Kieran M. McGrail, bs[2]
Theresa M. Sumrall, bs[2]
John R. Wingard, md[3]
Douglas W. Theriaque, ms[4]
Jonathan J. Shuster, phd[4]
Mark A. Atkinson, phd[2]
Desmond A. Schatz, md[1]

OBJECTIVE—We conducted an open-label, phase I study using autologous umbilical cord blood (UCB) infusion to ameliorate type 1 diabetes (T1D). Having previously reported on the first 15 patients reaching 1 year of follow-up, herein we report on the complete cohort after 2 years of follow-up.

RESEARCH DESIGN AND METHODS—A total of 24 T1D patients (median age 5.1 years) received a single intravenous infusion of autologous UCB cells and underwent metabolic and immunologic assessments.

RESULTS—No infusion-related adverse events were observed. β-Cell function declined after UCB infusion. Area under the curve C-peptide was 24.3% of baseline 1 year postinfusion ($P < 0.001$) and 2% of baseline 2 years after infusion ($P < 0.001$). Flow cytometry revealed increased regulatory T cells (Tregs) ($P = 0.04$) and naive Tregs ($P = 0.001$) 6 and 9 months after infusion, respectively.

CONCLUSIONS—Autologous UCB infusion in children with T1D is safe and induces changes in Treg frequency but fails to preserve C-peptide.

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Autologous umbilical cord blood (UCB) is an attractive source for potential cell therapies in young children. On the basis of preclinical efficacy and safety data, we performed an unblinded observational pilot study to determine whether autologous UCB infusion could preserve remaining endogenous insulin production.

RESEARCH DESIGN AND METHODS

—Subjects >1 year of age, with type 1 diabetes (T1D), and for whom autologous UCB was stored, were recruited for participation (clinical trial reg. no. NCT00305344; FDA IND BB-11918). A detailed description of the study, as well as results from the first 15 subjects to reach 1 year of postinfusion follow-up, was previously published (1).

Procedures

Peripheral blood and an aliquot of UCB from potential subjects were shipped to the University of Florida for infectious disease testing, HLA confirmation, and viability screening. Thereafter, the UCB unit of qualified subjects was shipped to the University of Florida and stored until transfused. Subjects underwent a baseline 2-h mixed meal tolerance test and had blood drawn for complete blood count, basic metabolic panel, HbA_{1c}, and flow cytometry, with staining for CD3, CD4, CD8, CD25, CD62L, CD45RA, and FOXP3 (2–4). UCB was thawed and washed per standard operating procedures. Subjects received pretreatment with diphenhydramine and acetaminophen. No other preparative therapy was given. Thawed UCB cells were infused through a peripheral IV over 10–20 min. Subjects returned for follow-up testing (identical to baseline) every 3 months in the 1st postinfusion year and every 6 months in the 2nd postinfusion year.

Statistical analysis

To determine changes from baseline, we calculated fractional change in area under the curve (AUC) C-peptide for each subject as $[(Y_n/Y_0) - 1]$, where the subscript n is the month number. Fractional changes were tested for a target population null hypothesis of a median of 0 by the two-sided Wilcoxon rank-sum test, a nonparametric procedure. Slope analysis was performed to calculate the rate of decline in AUC C-peptide over time. The pilot nature of this study dictated against controlling study-wide errors via either a Bonferroni correction or formal multivariate analysis. Because of concern for outliers, descriptive statistics are presented as median (quartiles).

RESULTS

—Between 15 December 2003 and 21 November 2008, 24 children with T1D (10 males, 14 females) underwent a single autologous UCB transfusion. No adverse events were observed in association with autologous UCB infusion. All aliquots of UCB had negative Gram stains, and none grew pathogenic organisms when cultured for virus, bacteria, or fungus.

Baseline and longitudinal postinfusion characteristics are provided in Table 1. Median age at infusion was 5.1 years (3.4–6.9). Median time from diagnosis to UCB infusion was 0.25 years (0.19–0.52). The median infused total nucleated cell count was 1.88×10^7 cells/kg. Median viability was 97% (95–99%). Overall, the total nucleated cell count recovered was

From the [1]Department of Pediatrics, University of Florida, Gainesville, Florida; the [2]Department of Pathology, University of Florida, Gainesville, Florida; the [3]Department of Medicine, University of Florida, Gainesville, Florida; and the [4]Department of Epidemiology and Health Policy Research and the Clinical and Translational Science Institute, University of Florida, Gainesville, Florida.
Corresponding author: Michael J. Haller, hallemj@peds.ufl.edu.
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Table 1—*Baseline and postinfusion characteristics of autologous UCB recipients*

	Preinfusion	Postinfusion						(Preinfusion–to–1 year ratio) − 1	(Preinfusion–to–2 year ratio) − 1
		3 months	6 months	9 months	1 year	1.5 years	2 years		
HbA_{1c} (%)	7.4	7.5	7.5	7.1	7.1	7.1	7.6	−0.2	0.1
	(6.5–8.4)	(7.1–8.5)	(7.1–8.5)	(6.7–7.1)	(6.7–7.8)	(6.6–7.9)	(7.2–8.0)	(−0.6 to 0.7)	(−0.5 to 0.9)
	{24}	{24} [NS]	{23} [NS]	{23} [NS]	{24} [NS]	{20} [NS}	{21} [NS]	{24} [NS]	{21} [NS]
Insulin use									
(units/kg per day)	0.37	0.46	0.58	0.58	0.69	0.63	0.66	−0.22	−0.44
	(0.22–0.51)	(0.28–0.58)	(0.44–0.79)	(0.44–0.79)	(0.55–0.81)	(0.57–0.77)	(0.61–0.81)	(−0.53 to −0.085)	(−0.68 to −0.21)
	{24}	{23} [NS]	{23} [NS]	{23} [NS]	{23} [<0.001]	{20} [0.001]	{21} [<0.001]	{20} [<0.001]	{21} [<0.001]
Peak C-peptide									
(ng/mL)	1.16	0.83	0.73	0.6	0.28	0.17	0.05	−0.53	−0.7
	(0.7–1.71)	(0.24–1.28)	(0.47–1.35)	(0.08–1.36)	(0.16–0.86)	(0–0.95)	(0–0.58)	(−1.0 to −0.16)	(−1.4 to −0.5)
	{22}	{24} [0.006]	{23} [0.003]	{20} [<0.001]	{21} [<0.001]	{19} [<0.001]	{21} [<0.001]	{20} [<0.001]	{19} [<0.001]
AUC C-peptide									
(ng/mL)	0.95	0.66	0.64	0.46	0.22	0.15	0.02	−0.49	−0.6
	(0.51–1.4)	(0.2–1.0)	(0.2–1.1)	(0.03–1.1)	(0.02–0.63)	(0–0.7)	(0–0.52)	(−0.82 to −0.11)	(−1.0 to −0.37)
	{22}	{24} [0.002]	{23} [<0.001]	{20} [<0.001]	{21} [<0.001]	{21} [<0.001]	{21} [<0.001]	{21} [<0.001]	{19} [<0.001]
IA-2A	11.0	7.5	4.5	4.1	2.9	2.2	2.3	−3.8	−7.2
	(1.8–23.1)	(0.69–20)	(0.2–16.3)	(0–16.5)	(0–14.5)	(0–9.9)	(0–8.9)	(−7.5 to −0.35)	(−12.7 to −2.3)
	{22}	{21} [0.01]	{22} [0.01]	{20} [NS]	{21} [0.001]	{18} [<0.001}	{18} [<0.001]	{20} [0.001]	{16} [<0.001]
GADA	2.5	1.7	1.95	2.0	2.6	1.9	1.2	−1.0	−0.8
	(0.7–12.3)	(0.3–6.4)	(0.2–9.2)	(0.5–15.7)	(0.3–15.8)	(0.4–5.3)	(0.2–8.9)	(−3.6 to 0.6)	(−8.4 to 0)
	{23}	{21} [NS]	{22} [NS]	{20} [NS]	{22} [NS]	{18} [0.01]	{19} [NS]	{22} [NS]	{18} [NS]
WBC (cell × 10^9/L)	5.6	5.75	6.1	5.8	5.5	5.8	5.5	−0.4	−0.5
	(5.0–7.3)	(4.7–7.85)	(4.7–6.9)	(4.6–6.9)	(4.4–6.7)	(3.9–7.6)	(4.25–7.35)	(−0.7 to 0.2)	(−1.8 to 0.6)
	{23}	{24} [NS]	{23} [NS]	{20} [NS]	{22} [NS]	{18} [NS]	{20} [NS]	{22} [NS]	{19} [NS]
CD4-to-CD8 ratio	1.89	1.89	1.89	1.96	1.97	1.90	2.15	0.1	0.15
	(1.5–2.4)	(1.6–2.2)	(1.5–1.9)	(1.6–2.2)	(1.7–2.2)	(1.4–2.1)	(1.7–2.4)	(−0.28 to −0.24)	(−0.2 to −0.4)
	{22}	{23} [NS]	{23} [NS]	{20} [NS]	{22} [NS]	{18} [NS]	{20} [NS]	{21} [NS]	{18} [NS]
Peripheral blood									
Treg (%)	3.1	4.1	4.4	3.6	4.4	3.0	3.3	0.13	0.52
	(0.8–5.4)	(1.0–5.5)	(2.0–7.5)	(1.9–5.1)	(2.0–7.6)	(1.8–5.1)	(1.9–6.5)	(−1.3 to 1.0)	(−1.8 to 2.9)
	{22}	{23} [NS]	{23} [0.04]	{20} [NS]	{22} [NS]	{20} [NS]	{20} [NS]	{21} [NS]	{18} [NS]
CD45RA									
Treg (%)	39.0	42.6	40.5	43.5	40.9	40.2	42.8	2.24	1.82
	(25.7–45.9)	(27.8–49.8)	(34.1–51.3)	(37.9–54.3)	(31.9–50.4)	(29.9–46.2)	(28.6–48.8)	(−5.53 to 11.0)	(−3.8 to 1.8)
	{22}	{23} [NS]	{23} [NS]	{20} [0.001]	{22} [NS]	{18} [NS]	{20} [NS]	{21} [NS]	{18} [NS]

Data are median (interquartile range) and {*n*} [*P* value vs. baseline]; *N* = 24 (10 males, 14 females), median age at infusion 5.1 years (3.4–6.9). IA-2A, insulinoma-associated 2 antibody; GADA, GAD antibody; WBC, white blood cell count.

commonly 1–2 log fold less than that typically observed in samples obtained from public banks (5).

Median AUC C-peptide at the time of autologous UCB infusion was 0.95 ng/mL (0.5–1.4). Median AUC C-peptide declined at all subsequent study visits compared with baseline ($P < 0.01$ at all time points) (Table 1). At post-UCB infusion visits at 3, 6, and 9 months and 1 and 2 years, median AUC C-peptide was 0.66 ng/mL (0.2–1), 0.64 ng/mL (0.2–1.1), 0.46 ng/mL (0.03–1.1), 0.22 ng/mL (0.02–0.63), and 0.02 ng/mL (0–0.52), respectively (Supplementary Fig. 1). AUC C-peptide was 24.3% of baseline at 1 year after infusion and 2% of baseline 2 years after infusion. Slope analysis of C-peptide demonstrated AUC C-peptide change of −2.4% per month (−3.09 to −1.43) ($P < 0.001$).

Baseline and 1- and 2-year median total peripheral white blood counts were 5.6×10^9, 5.5×10^9, and 5.5×10^9 cells/L, respectively (not significant vs. baseline). Total T-cell numbers, CD4-to-CD8 ratio, $CD62L^+$ regulatory T cells (Tregs), $CD62L^+$ T effectors, $CD45RA^+$ T conventional cells, and $CD45RO^+$ Tregs were not different at all study visits compared with baseline (data not shown). However, an increase in total Treg ($CD4^+CD25^+FOXP3^+$) was observed at the 6-month visit (median 4.4%, $P = 0.04$), as was an increase in $CD45RA^+$ Treg at the 9-month visit (median 43.5%, $P = 0.001$) (Table 1).

CONCLUSIONS—A single infusion of minimally manipulated autologous UCB in young children with T1D is feasible and safe but fails to preserve C-peptide. Lack of control subjects (in this case, attributable to internal review board and U.S. Food and Drug Administration restrictions) makes it difficult to form definitive conclusions regarding efficacy. The observation that total Treg frequency was increased up to 6 months after infusion suggests that autologous UCB infusion may favorably alter the T-cell repertoire in children with T1D.

The reasons for an inability of autologous UCB to effectively halt autoimmune progression are at least twofold. First, an insufficient number of cells carrying regenerative or immunoregulatory capacity may have been transferred to patients with T1D. In addition, the ongoing autoimmune response in new-onset T1D subjects may contain memory T cells, refractive to regulation by Tregs (6), that facilitate the ongoing autoimmune destruction of endogenous or de novo β-cells.

To address the first issue, efforts are underway to isolate and expand specific cell populations within UCB to augment their therapeutic potential. As proof of concept, a phase I clinical trial is currently under way in adult patients with recent-onset T1D using autologous expanded Tregs isolated from peripheral blood (clinical trial reg. no. NCT01210664). In terms of the second limitation, studies from our laboratory suggest that a combination therapeutic approach involving transient immune depletion and subsequent induction of immune regulation is optimal (7). As such, we believe that therapies combining transient immune depletion and subsequent infusion of expanded UCB Tregs may more effectively reset the balance of Tregs and effector T cells in T1D.

Although this effort failed to demonstrate benefit, the potential of UCB to participate in future T1D interventional therapies remains. Efforts to use autologous UCB in the treatment of T1D will continue with emphasis on improved understanding of UCB Treg function, the addition of generally regarded as safe therapies (i.e., vitamin D and n-3 fatty acids) to UCB infusion (clinical trial reg. no. NCT00873925), and perhaps most important, the potential use of expanded autologous UCB Tregs either alone or in combination with other immunomodulatory agents.

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