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Gene products promoting remyelination are up-regulated in a cell therapy product manufactured from banked human cord blood

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Abstract

Background aims. DUOC-01, a cell product being developed to treat demyelinating conditions, is composed of macrophages that arise from $CD14^+$ monocytes in the mononuclear cell (MNC) population of banked cord blood (CB). This article demonstrates that expression of multiple gene products that promote remyelination is rapidly up-regulated during manufacturing of DUOC-01 from either MNC or purified CB $CD14^+$ monocytes. *Methods.* Cell cultures were initiated with MNC or with immunoselected $CD14^+$ monocytes isolated from the same CB unit. Cell products present in these cultures after 2 and 3 weeks were compared by three methods. First, quantitative polymerase chain reaction was used to compare expression of 77 transcripts previously shown to be differentially expressed by freshly isolated, uncultured CB $CD14^+$ monocytes and DUOC-01. Second, accumulation of 16 soluble proteins in the culture medium was measured by Bioplex methods. Third, whole transcriptomes of the cell products were compared by microarray analysis. *Results.* Key transcripts in multiple pathways that promote remyelination were up-regulated in DUOC-01, and substantial secretion of proteins corresponding to many of these transcripts was detected. Cell products manufactured from MNC or from $CD14^+$ monocytes were similar with regard to all metrics. Upregulation of gene products characteristic of DUOC-01 was largely completed within 14 days of culture. *Conclusion.* We demonstrate that expression of multiple gene products that promote remyelination is up-regulated during the first 2 weeks of manufacturing of DUOC-01. Measuring these mechanistically important transcripts and proteins will be useful in monitoring manufacturing, evaluating manufacturing changes, and developing mechanism-based product potency assays.

Key Words: *cord blood, macrophage, monocytes, remyelination*

Introduction

Circulating monocytes, macrophage and brain microglia all regulate myelination following brain injury [1–7]. DUOC-01, a cell therapy product intended for use in treatment of demyelinating conditions, is composed of cells resembling macrophage in morphology, phagocytic activity and antigen expression [8,9]. A phase I trial (ClinicalTrials.gov identifier NCT02254863) exploring the safety and feasibility of using intrathecally administered DUOC-01 in the clinical setting of allogeneic cord blood (CB) transplantation for inherited metabolic diseases is currently enrolling subjects.

DUOC-01 is manufactured from banked, volume- and red cell–reduced, mononuclear cell (MNC)-enriched human CB. During the 21-day manufacturing process, most of the initiating MNCs die, and an adherent, phagocytic, motile cell population emerges as the final cell product [8]. Intracerebral injection of this product accelerates remyelination of the brain corpus callosum of mice after cuprizone feeding [9]. Available evidence suggests that the cells in DUOC-01 are derived from $CD14^+$ monocytes in the MNC population used to initiate cultures. However, CB $CD14^+$ monocytes and the cells in DUOC-01 differ greatly in gene expression. Significantly, expression of many genes that promote myelination is up-regulated in DUOC-01 relative to CB $CD14^+$ monocytes, and DUOC-01 promotes remyelination more efficiently than uncultured $CD14^+$ monocytes in the cuprizone model [9]. We have identified several proteins that are highly expressed by DUOC-01 and that may promote remyelination by different mechanisms. Thus, cells in DUOC-01 secrete anti-inflammatory (interleukin [IL]-10) and neurotrophic (IL-6) cytokines, matrix

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remodeling proteases (MMP12), and strongly express the myelin degradation sensing receptor TREM2 that signals to reparative glial cells following demyelination. DUOC-01 also secretes cytokines that drive the differentiation of oligodendrocytes (KITLG, IGF1, PDGFα), and treatment with DUOC-01 strongly increases the number of dividing and differentiating oligodendrocyte progenitors in the cuprizone model [8,9]. These findings are consistent with the hypothesis that multiple mechanisms of remyelination are activated in CB CD14$^+$ monocytes as they give rise to DUOC-01 during manufacturing and support the use of DUOC-01 to treat demyelinating diseases.

In this article, we provide additional data showing that expression of many molecules that can enhance remyelination is up-regulated during manufacture of DUOC-01. Building on previous microarray results [9], we selected a group of transcripts for genes that regulate myelination and, in the work described here, used quantitative polymerase chain reaction (qPCR) methods to confirm that these genes are highly expressed by DUOC-01. These transcripts include myelination-promoting gene products we have previously shown to be expressed by DUOC-01 and also additional gene products in related and different mechanistic pathways. We also demonstrate that DUOC-01 cells secrete 16 proteins potentially relevant to brain repair. We studied the time course of protein secretion and of changes in expression of transcripts characteristic of DUOC-01 as ways to monitor the progress of the manufacturing process. Finally, we initiated cell cultures as usual with CB MNCs and also in parallel with CB CD14$^+$ cells isolated from the same cord, carried the both sets of cultures through the standard DUOC-01 manufacturing process and assessed the similarity of the resulting cell products by qPCR, by measuring accumulation of secreted proteins and by performing whole transcriptome microarray analysis. We report that the cell products derived from either starting population are highly similar, a result consistent with the idea that CD14$^+$ monocytes give rise to the DUOC-01 product. The results highlight several important mechanisms through which DUOC-01 can accelerate remyelination and provide the basis for in-process testing, manufacturing comparability studies and definitive mechanism-based product potency assays for this cell product.

Methods

Manufacturing cell products

The basic strategy for these experiments is diagramed in Figure 1. Volume-reduced, red cell–reduced and MNC-enriched cryopreserved CB units were obtained from the Carolinas Cord Blood Bank, a U.S. Food and Drug Administration–licensed public cord blood bank at Duke University Medical Center. This material had been collected from donors who had given informed consent for samples to be used for research purposes under local institutional review board–approved protocols. Each unit was thawed and was used to manufacture DUOC-01 from MNC populations as previously described [8]. A portion of the MNC population was reserved and used to purify CD14$^+$ monocytes using immunomagnetic methods [9], and these CD14$^+$ cells were cultured using the identical manufacturing process yielding CD14$^+$ monocyte-derived product. Cells and culture

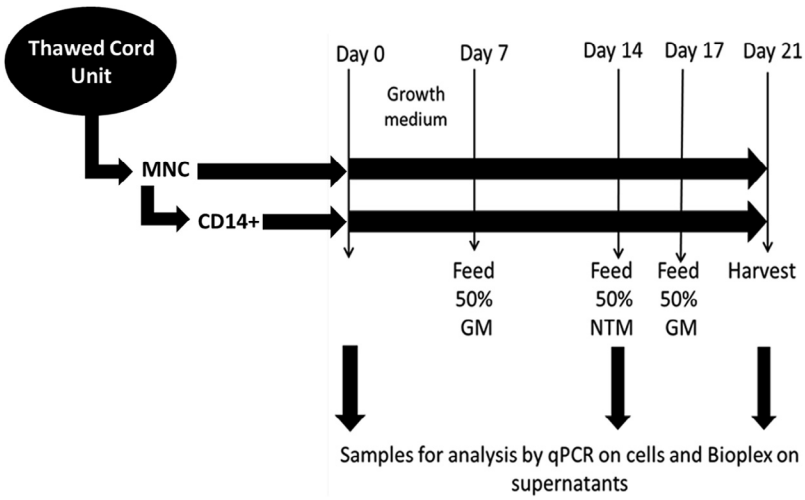

Figure 1. Diagram of experimental design described in the text. GM and NTM are the growth medium and neurotrophic medium described by Kurtzberg *et al.* [8]. Day 0 cells were not cultured. Day 14 samples were from cells that were cultured in GM only and had not been exposed to NTM medium. Day 21 cells were cultured in 50% NTM medium/50% GM for 3 days and then 25% NTM/75% GM medium for 4 days. The same protocol was used to manufacture cells for whole transcriptome analysis.