Cytotherapy, 2017; 19: 771–782

International Society for Cellular Therapy
ISCT®

CrossMark

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

PAULA SCOTLAND, SUSAN BUNTZ, PAMELA NOELDNER, ARJUN SAHA, TRACY GENTRY*, JOANNE KURTZBERG & ANDREW E. BALBER†

Robertson Clinical and Translational Cell Therapy Program, Duke Translational Medicine Institute, Duke University Medical Center, Durham, North Carolina, USA

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

*Current affiliation: Duke Clinical Research Institute, Durham, North Carolina, USA.
†Current affiliation: Cicada Consulting, Durham, North Carolina, USA.
Correspondence: **Andrew E. Balber**, PhD, Cicada Consulting, 2608 Nation Avenue, Durham, NC 27707, USA. E-mail: andrew.balber@duke.edu

(Received 15 February 2017; accepted 2 March 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.03.004

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

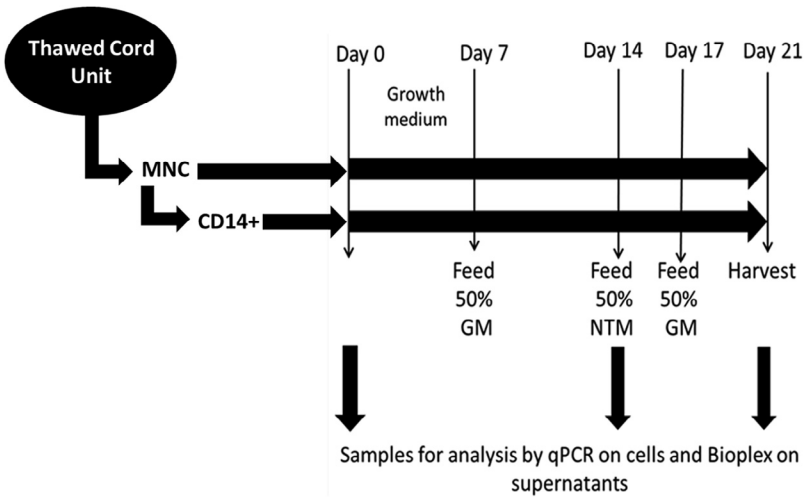


Figure 1. Diagram of experimental design described in the text. GM and NTM are the growth medium and neurotrophic medium described by Kurtzberg *et al.* [8]. Day 0 cells were not cultured. Day 14 samples were from cells that were cultured in GM only and had not been exposed to NTM medium. Day 21 cells 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.

supernatants were harvested at various times during the manufacturing process to perform the assays described subsequently.

Quantitative polymerase chain reaction

Quantitative polymerase chain reaction was used to measure levels of transcripts in individual genes or in a custom array (Qiagen) using proprietary primers as previously described [9]. Total RNA was isolated using RNeasy Mini Kit with DNase1 treatment. For custom arrays, samples were prepared from 400 ng of total RNA using the RT^2 First Strand kit, followed by the RT^2 Profiler PCR Array kit as instructed by the manufacturer. Custom arrays, RNeasy Mini Kit, RT^2 First Strand Kit, and RT^2 Profiler PCR Array Kit were obtained from Qiagen (Germantown). For analysis of individual genes, equal amounts of RNA was used in cDNA synthesis using Superscript III polymerase with oligo (dT) primers as instructed (Life Technologies). Diluted cDNA was amplified on the BioRad CFX96 RealTime System using SsoAdvanced Universal SYBR Green Supermix (BioRad) using the following oligonucleotides: MMP9 (sense 5′- TGTACCGCTATGGTTACACTCG-3′, antisense 5′- GGCAGGGACAGTTGCTTCT-3′), MMP-12 (sense 5′-CAAAACTCAAATTGGGG TCACAG-3′, antisense 5′-CTCTCTGCTGATGACA TACGTG-3′), TREM-1 (sense 5′-TGCCCCTCTTT CAGTTCATAC-3′, antisense 5′-CACAGGAAGGATG AGGAAGAC-3′), TREM-2 (sense 5′-TCATAGGGG CAAGACACCT-3′, antisense 5′-GCTGCTCATC TTACTCTTTGTC-3′), SCF/KITLG (sense 5′-AGCTGAAGATAAATGCAAGTGAG-3′, antisense 5′-CAGAACAGCTAAACGGAGTCG-3′) and VEGFa (sense 5′-TGGTGACATGGTTAATCGGT-3′, antisense 5′-AGAAAGACAGAACAAAGCCAGA-3′). ΔCt values were normalized to glyceraldehyde 3-phosphate dehydrogenase expression. ΔΔCt values were determined in relation to freshly isolated CB CD14$^+$ monocytes.

Accumulation of human proteins in culture medium

The concentrations of 16 secreted proteins in supernatants removed from cultures initiated with CB MNC or with purified CD14$^+$ monocytes derived from the same cord were compared. Supernatants were collected before feeding on day 14 and during harvesting of final cell products on day 21. Secreted protein concentrations were measured [8] by antibody capture immunoassay with fluorescence reporters using the Bio Rad Bioplex 200 instrument. IL-6, IL-10 and 10 chemokines were multiplexed on one plate (Biorad catalog no. 171-AK99MR2, standard lot no. 5036571), four human matrix metalloproteases on a second (catalog no. 171-AM001M, standard lot no. 5042979, and MIP-1β was assayed on the third (catalog no. 171-D50001, standard lot no. 5039890). Standards for each protein provided by the manufacturer were diluted in appropriate uninoculated tissue culture medium to construct standard curves, and the concentration of each protein in the supernatants was calculated.

Microarray analysis

Three CB units were split to manufacture DUOC-01 from CB MNCs and cell products from CD14$^+$ monocytes as described above. RNA was isolated from each product using the Qiagen RNeasy kit as described by the manufacturer and used for whole genome microarray analysis. Microarray analysis was performed [9] by the Microarray Shared Resource in the Duke Center for Genomic and Computational Biology using Affymetrix GeneChip Human Transcriptome Array 2.0 microarrays. Partek Genomics Suite 6.6 (Partek) was used to perform data analysis. Robust multichip analysis normalization was done on the entire data set. Multi-way analysis of variance and fold change were performed to select target genes that were differentially expressed. Hierarchical clustering was performed on differentially expressed genes based on average linkage with Pearson's dissimilarity.

Results

General strategy and pilot analysis of kinetics of gene regulation during manufacturing

The primary goal of these studies was to gather more information related to possible mechanisms of action of DUOC-01 cells in brain remyelination. In addition, we wished to develop probes that reflect expression of mechanistically important gene products to monitor manufacturing of this cell product, assess activity and product potency and determine product comparability following manufacturing changes.

To control for inherent biological variability among CB units, we opted to compare cell products made from portions of the same CB unit under different conditions rather than compare products made from different units during this manufacturing-oriented work. This placed limits on the number of variables we could explore with a single unit. Consequently, before proceeding with full scale studies, we first performed a small pilot experiment to determine how rapidly characteristic changes in gene expression could be detected during the DUOC-01 manufacturing process and whether these changes followed the same kinetics in cultures initiated with CD14$^+$ monocytes instead of MNC. On the basis of our previous morphological studies of DUOC-01 in culture [8] and the yield of cells derived at various times during manufacturing, we decided to begin our analysis on day 14

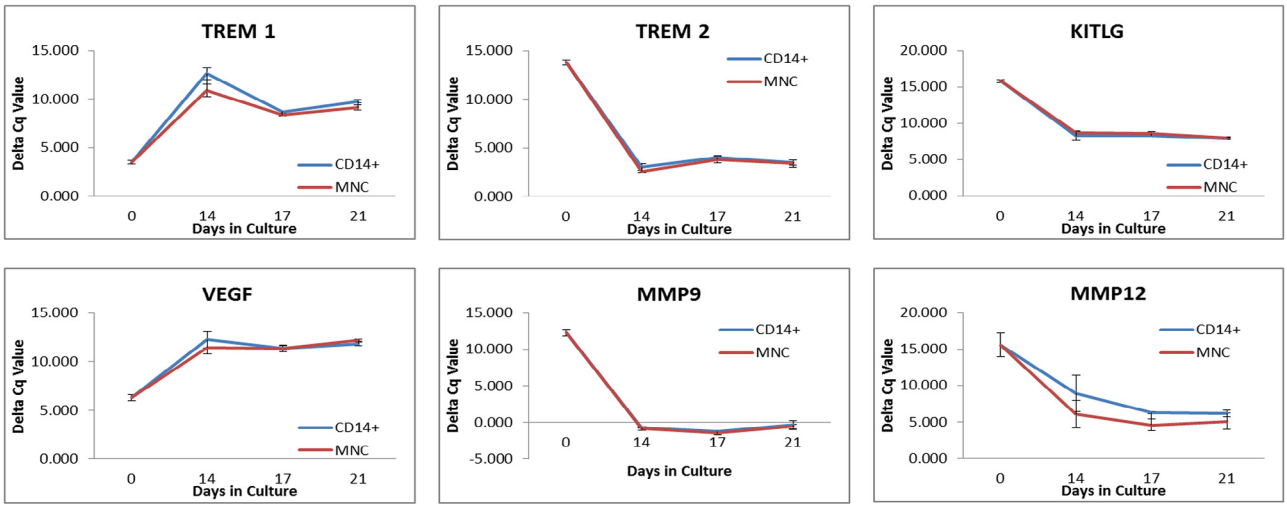


Figure 2. Changes in expression of selected transcripts during manufacture of cell products from cord blood CD14$^+$ monocytes and cord blood mononuclear cells. Cultures were initiated with either cell population as described in the text, and cell products were harvested on the days shown and analyzed by qPCR for expression of the genes indicated. Each time point shows the mean ± SEM ΔCq value normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for experiments done with three CB units. Increase in ΔCq indicates a decrease in transcript abundance, and decrease indicates an increase in abundance relative to GAPDH.

of manufacturing just before the first medium change, on day 17 just before the medium was changed again, and when the cell product was harvested on day 21 (Figure 1). We used qPCR to analyze expression of six genes that were differentially expressed in our previous microarray studies demonstrating that TREM1 and VEGF were down-regulated in cultured DUOC-01 cell products relative to freshly isolated CD14$^+$ monocytes, and TREM2, KITLG, MMP9 and MMP12 were up-regulated [9]. The results (Figure 2) confirmed these changes in the abundance of transcripts for the six genes. In addition, Figure 2 shows that medium changes on days 14 and 17 during manufacturing had no detectable effect on the abundance of these six transcripts. Regulation of all six gene products appeared to be complete by day 14 of manufacturing whether cultures were started with MNCs or with CD14$^+$ monocytes. We therefore opted to compare only the activities of cell products cultured for 14 and 21 days in subsequent full-scale studies. Thus, we did experiments in which gene expression by four cell products—days 14 and 21 cultures derived from either CB MNC or from CD14$^+$ monocytes—from the same CB unit were compared.

Expression of 77 transcripts related to remyelination during the manufacturing process

To measure expression of gene products that can contribute to remyelination by DUOC-01, we constructed a custom array for qPCR analysis. We used our previous microarray analysis to guide probe selection. Many of the transcripts we selected have multiple biological activities, but all represent potentially important activities in pathways that modulate neural or glial cell activity during brain repair or development. Supplementary Material 1 lists the genes assayed in the array and abbreviations. The array included 24 gene products that we expected to be uniquely expressed by DUOC-01 on the basis of our previous microarray study. We were particularly interested in those transcripts because these genes could be responsible for previously described [9] enhanced remyelinating activity of DUOC-01 compared with CD14$^+$ monocytes. However, genes that are expressed by both DUOC-01 and CD14$^+$ monocytes could also participate in remyelination, and we included 45 probes for such genes. Finally, we added probes for eight genes that we expected to be strongly expressed by CD14$^+$ monocytes but not detectably expressed by DUOC-01. This allowed us to monitor extinction of transcripts characteristic of CB CD14$^+$ cells as well as appearance of transcripts typical of DUOC-01 for in-process monitoring and other testing related to quality and regulatory purposes. We used this custom array to measure expression of these 77 genes by cells in DUOC-01, that is, product manufactured from CB MNC, using the standard 21-day protocol. We also compared gene expression by these standard MNC-derived DUOC-01 products to CB CD14$^+$ monocyte-derived cell products from the same three cord blood units harvested after 14 and 21 days in culture.

Raw Ct data for expression of transcripts detected by each of the 77 probes during the three manufacturing runs using are presented in Supplementary Material 2. Changes in expression calculated

from these data are summarized in Figure 3. We defined the limit at which we could reliably detect expression at 35 cycles. Thus, any value of Ct > 35 is considered not expressed, and any value of ΔCt calculated using a Ct value > 35 represents a lower limit of changes in expression. In general, changes in gene expression in cells derived from all CB units changed in the same way; changes in CCL13, CXCL12, C1QC and IGF1, showed the most variability between units after 21 days in culture. As expected, 24 genes in the custom array were not detectably expressed by freshly isolated CB CD14$^+$ monocytes genes (Group A in Figure 3), and eight were not detectably expressed by DUOC-01 (Group C). Forty genes (Group B) were detectably, but differentially, expressed by the two cell types. Within Group B, 25 genes were more highly expressed in DUOC-01, and 15 were more highly expressed in non-cultured CD14$^+$ monocytes. The degree of over-expression ranged from about 2-fold to more than 30,000-fold. Five genes (Group D) were either expressed at very low levels or were not differentially expressed. With the exception of the transcripts in Group D, the results in Figure 3 generally confirm the expression differences detected by the microarray chip and allow assignment of discrete gene expression profiles to DUOC-01 cells and to the CD14$^+$ monocytes from which DUOC-01 cells are derived during manufacturing.

Figure 3 also shows that DUOC-01 (blue) and CD14$^+$ monocyte–derived (red) cell products harvested after 21 days in culture were highly similar with respect to gene expression profile. Cells analyzed after 14 days in culture (left data column for each gene in Figure 3) had already changed in expression of each transcript analyzed relative to freshly isolated CD14$^+$ cells, and little or no change in transcript abundance was detected when cells were cultured for another week (right data column for each gene). This extends the results presented for six genes in Figure 2 to the full 77-gene custom array.

Accumulation of secreted proteins in supernatants during manufacturing

To determine how these gene expression patterns reflect production of proteins, we used Bioplex methods to measure accumulation of IL-6, IL-10 and 10 chemokines in the medium of cultures initiated with CB MNC or CB CD14$^+$ monocytes. These culture supernatants were collected from the same cultures that were analyzed by qPCR; results are presented in Figure 4. We previously reported that IL-10 and IL-6 accumulated in significant amount in culture medium harvested on day 21 [8]; we confirmed this result in these three additional manufacturing batches. In addition, we found that IL-10 could be readily detected in medium on day 14. IL-6 was present in very low concentrations at this time (Figure 4, bottom left). We note that qPCR data in Figure 3 indicates that IL-6 and IL-10 are somewhat more highly expressed at the transcript level by CD14$^+$ monocytes than by DUOC-01 cells. Nevertheless, both proteins were actively produced by DUOC-01.

All 10 chemokines could be detected in the medium on days 14 and 21 of the manufacturing process. The concentration of chemokines that accumulated in the medium varied considerably (Figure 4). CCL2, CCL4, CCL22 (all over-expressed by DUOC-01 at the transcript level; see Figure 3) and CXCL8 (IL-8; more transcript expressed in CD14$^+$ monocytes, Figure 3) were present in ng/mL amounts. CCL20 was detected in the 1–20 pg/mL range, and the other chemokines accumulated in intermediate concentrations. CCL8 and CXCL12 transcripts were not differentially expressed by qPCR (Figure 3). Similar levels of each chemokine accumulated in the medium of DUOC-01 and CD14$^+$ monocyte–derived cell products.

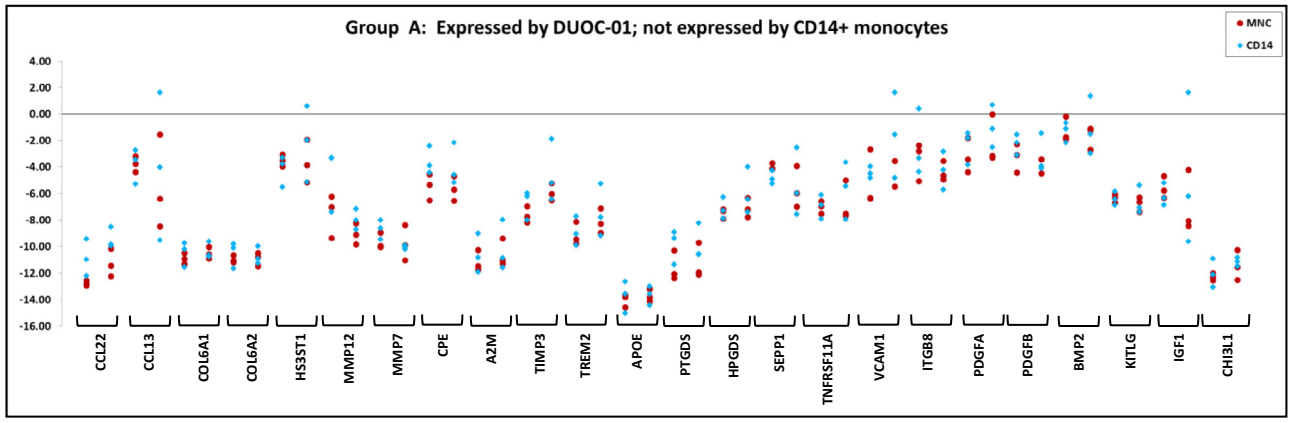
Figure 4 (bottom right) shows that four matrix metalloproteases accumulated at between 2.6 and 192 ng/mL amounts in the culture medium from DUOC-01 and CD14-derived cell products. MMP7, MMP9 and MMP12 were also included in the PCR array, and all three were over-expressed in DUOC-01 products (Figure 3). The amounts of proteases in cultures started with the two cell types were not statistically different. Again, accumulation of all of the proteins could be detected in supernatants by day 14 of manufacturing.

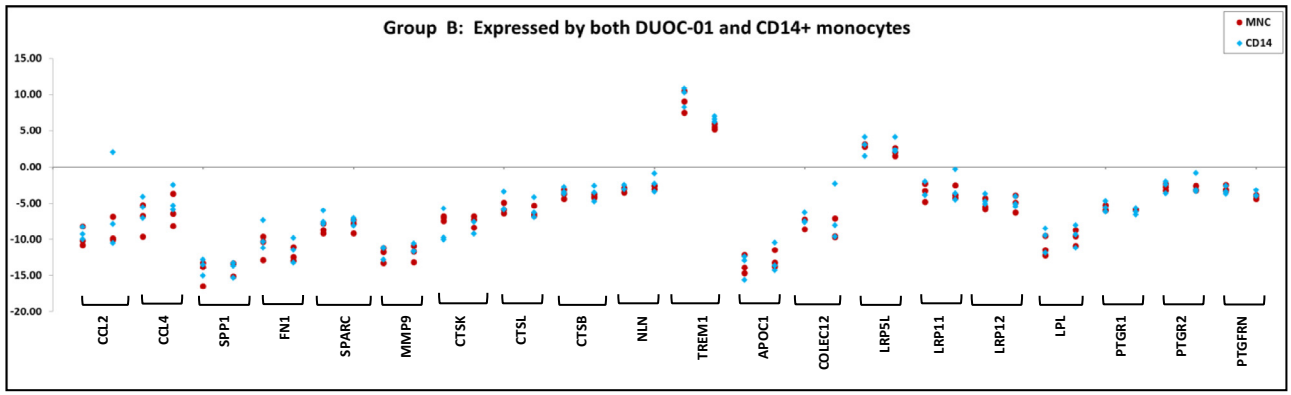
Comparison of transcriptomes of products manufactured from MNC and CD14$^+$ monocytes

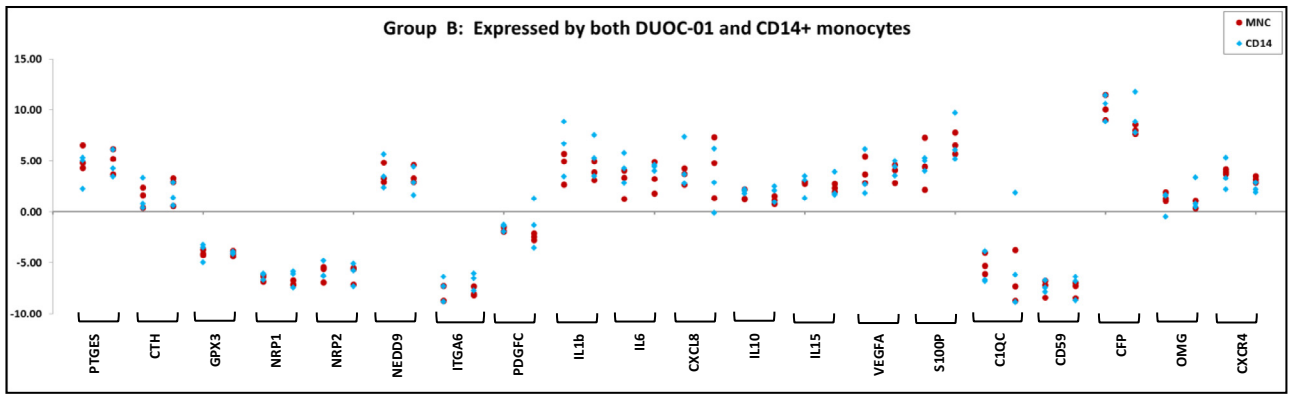
Finally, we used microarray methods to extend our expression analysis to the whole transcriptomes of DUOC-01 and of cell products manufactured from CB CD14$^+$ monocytes derived from the same CB units. Of the 54,675 transcripts probed on the whole transcriptome chip, only 24 showed a statistically significant ($P < 0.05$) difference in expression of more than twofold. These transcripts are listed in Table I. The differences in expression for these transcripts were all less than sixfold. We have not confirmed these expression differences by qPCR or protein expression methods.

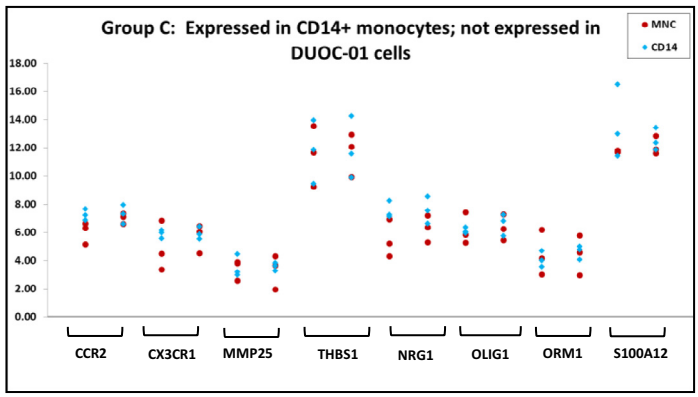
Discussion

The most important result of this study is the demonstration that multiple pathways that can promote brain myelination after injury are activated in cells in the standard DUOC-01 cell product that is currently used in clinical trials—the product initiated with


Group A: Expressed by DUOC-01; not expressed by CD14+ monocytes
MNC
CD14
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
-12.00
-14.00
-16.00
CCL22
CCL13
COL6A1
COL6A2
HS3ST1
MMP12
MMP7
CPE
A2M
TIMP3
TREM2
APOE
PTGDS
HPGDS
SEPP1
TNFRSF11A
VCAM1
ITGB8
PDGFA
PDGFB
BMP2
KITLG
IGF1
CHI3L1


Group B: Expressed by both DUOC-01 and CD14+ monocytes
MNC
CD14
15.00
10.00
5.00
0.00
-5.00
-10.00
-15.00
-20.00
CCL2
CCL4
SPP1
FN1
SPARC
MMP9
CTSK
CTSL
CTSB
NLN
TREM1
APOC1
COLEC12
LRP5L
LRP11
LRP12
LPL
PTGR1
PTGR2
PTGFRN


Group B: Expressed by both DUOC-01 and CD14+ monocytes
MNC
CD14
15.00
10.00
5.00
0.00
-5.00
-10.00
PTGES
CTH
GPX3
NRP1
NRP2
NEDD9
ITGA6
PDGFC
IL1b
IL6
CXCL8
IL10
IL15
VEGFA
S100P
C1QC
CD59
CFP
OMG
CXCR4


Group C: Expressed in CD14+ monocytes; not expressed in DUOC-01 cells
MNC
CD14
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
CCR2
CX3CR1
MMP25
THBS1
NRG1
OLIG1
ORM1
S100A12

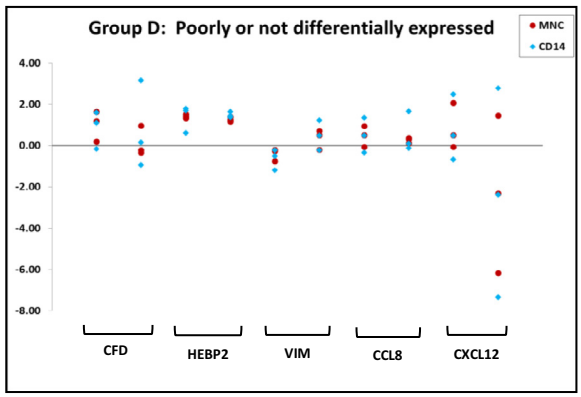

Group D: Poorly or not differentially expressed
MNC
CD14
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
CFD
HEBP2
VIM
CCL8
CXCL12

Figure 3. Changes in expression of 77 genes during manufacturing of cell products from CB MNC or CB CD14$^+$ monocytes. Cultures were initiated with CB MNC (red points) or CB CD14$^+$ monocytes (blue points). Cell products were harvested after 14 days (left column of data for each gene) or 21 days (right column of data) and analyzed by qPCR for expression of the genes indicated on the abscissa. Data points for both cell populations derived for each of three CB units are shown; some of these six points overlap in this format. The ordinate units are ΔΔCt value normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression in each sample and to expression by freshly isolated CD14$^+$ monocytes from the CB unit used to start the culture. Thus, positive values indicate that transcript is over-expressed in freshly isolated CD14$^+$ monocytes, and negative values mean that the transcript is over-expressed in the cultured cell population. The ordinate values are powers of 2; the line on each panel shows ΔΔCt = 0, or no change in expression relative to freshly isolated CD14$^+$ monocytes. The four groups are discussed in the text.

CB MNC and harvested after 21 days in culture. Of particular interest, we confirm here by qPCR methods that 24 transcripts not detectably expressed by freshly isolated, uncultured CB CD14$^+$ cells are highly expressed by DUOC-01 products. The genes represented by these transcripts are prime candidates for mediating the enhanced ability of DU0C-01 to accelerate remyelination when compared with CD14$^+$ monocytes in the cuprizone model. These transcripts also provide a characteristic expression profile for monitoring the DUOC-01 manufacturing process and potentially for measuring product potency. However, as CB CD14$^+$ monocytes also promote remyelination, albeit less extensively than DUOC-01 [9], gene products that are expressed by both DUOC-01 and by CB CD14$^+$ cells may also play a role in the mechanism of action and potency of the cell product.

To further elucidate these mechanisms, we have examined expression of proteins corresponding to several of the transcripts expressed strongly or uniquely by DUOC-01. This is important because we found previously [9], and also report here for the chemokines and metalloproteases, that relative transcript abundance does correlate with protein abundance in all

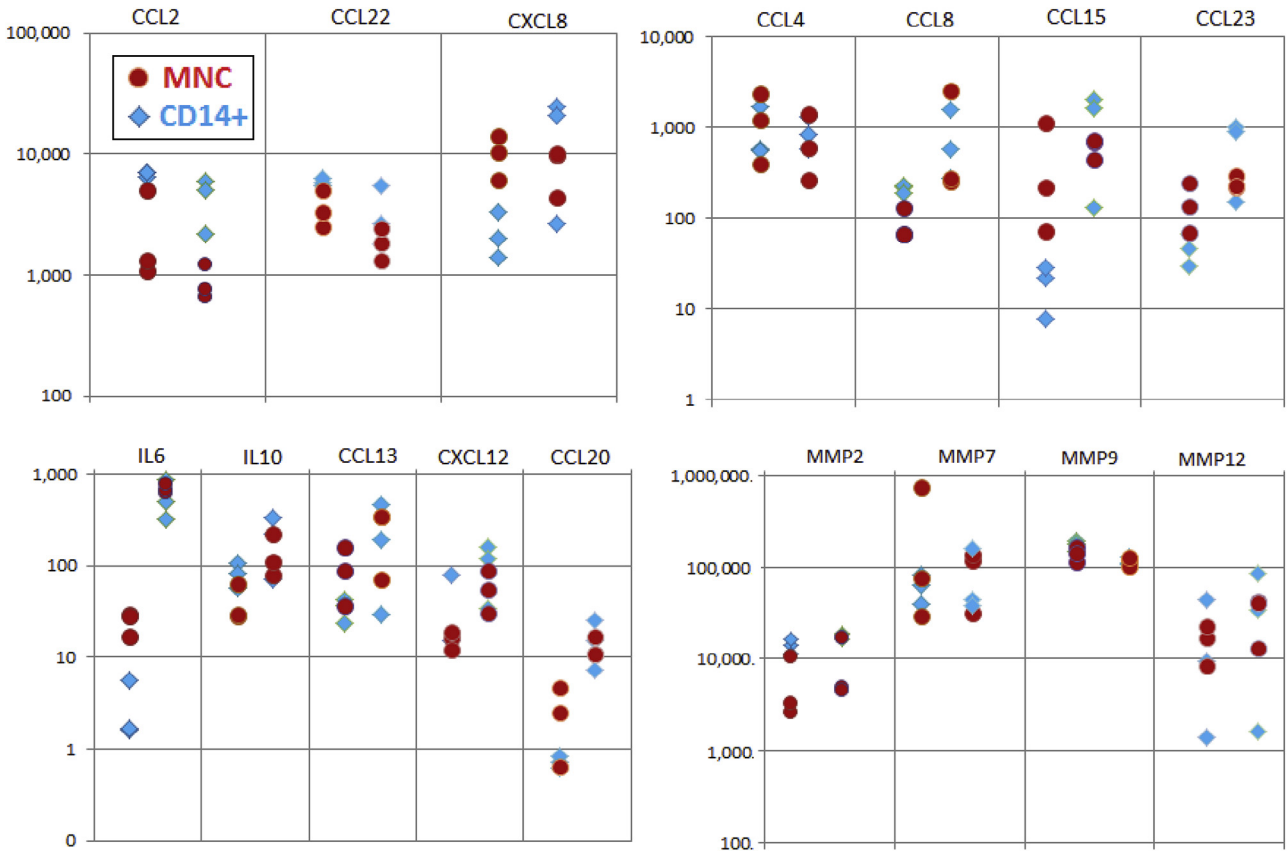


Figure 4. Concentration of chemokines, cytokines and metal metalloproteases accumulating in culture supernatants during manufacturing. Ordinate: picogram/milliliter measured by Bioplex; note that scales are different for each set of proteins. Abscissa: days in culture; for each protein, results for 14-day supernatants are plotted on the left, and 21-day supernatants, on the right. Data from three cords are shown; each point is the mean value for three analyses performed on an individual cell product. Blue points are data from cultures initiated with purified CB CD14$^+$monocytes. Red points are data from cultures initiated with CB mononuclear cells from the same cords. In the standard protocol for manufacturing DUOC-01, CB mononuclear cells are cultured for 21 days.

Table I. Microarray analysis of transcriptomes of cell product manufactured from MNC or CD14$^+$ monocytes from the same CB units.

Probeset ID	Gene symbol	Gene name	Fold Change	*P* value
205237_at	FCN1	ficolin (collagen/fibrinogen domain containing) 1	–5.87	0.019
203680_at	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	–4.03	0.028
209392_at	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	–3.72	0.014
210839_s_at	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	–3.22	0.039
213194_at	ROBO1	roundabout, axon guidance receptor, homolog 1 (Drosophila)	–2.76	0.047
209686_at	S100B	S100 calcium binding protein B	–2.58	0.042
220146_at	TLR7	toll-like receptor 7	–2.52	0.045
206111_at	RNASE2	ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)	–2.44	0.034
202478_at	TRIB2	tribbles homolog 2 (Drosophila)	–2.40	0.042
215838_at	LILRA5	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	–2.31	0.034
227677_at	JAK3	Janus kinase 3	–2.25	0.050
229934_at	—	—	–2.01	0.002
226869_at	MEGF6	multiple EGF-like-domains 6	2.00	0.028
203304_at	BAMBI	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	2.03	0.043
229125_at	KANK4	KN motif and ankyrin repeat domains 4	2.07	0.002
204879_at	PDPN	podoplanin	2.17	0.001
1559459_at	LOC613266	uncharacterized LOC613266	2.19	0.039
242871_at	PAQR5	progestin and adipoQ receptor family member V	2.26	0.011
219874_at	SLC12A8	solute carrier family 12 (potassium/chloride transporters), member 8	2.55	0.037
218469_at	GREM1	gremlin 1, DAN family BMP antagonist	2.88	0.022
209230_s_at	NUPR1	nuclear protein, transcriptional regulator, 1	2.98	0.017
203083_at	THBS2	thrombospondin 2	3.48	0.018
235874_at	PRSS35	protease, serine, 35	3.90	0.013
204475_at	MMP1	matrix metallopeptidase 1 (interstitial collagenase)	4.56	0.038

RNA prepared from each culture was subjected to microarray analysis. The table shows all transcripts differentially expressed (greater than twofold difference, $P < 0.05$, not corrected for multiple comparisons) by the two populations. Positive values indicate higher expression in cultures derived from CD14$^+$ monocytes.

cases. Taken together, our previous work [8,9] and this study show that DUOC-01 cells constitutively secrete the following proteins that can affect brain remyelination and repair in different clinical contexts: 10 lysosomal hydrolases, IL-6, IL-10, TGFB, PDGFA, IGF1, KITLG, MMP7, MMP9, MMP12, CCL2, CCL4, CCL8, CCL13, CCL15, CCL20, CCL22, CCL23, CXCL8 and CXCL12. In addition, DUOC-01 cells also express receptors that, through signaling pathways, can generate additional downstream effectors affecting brain repair. At the protein level, these include several membrane proteins widely expressed by macrophage and also TREM2, a receptor with particularly important functions in brain macrophage. Several potential networks for enhancing remyelination are implied by these transcript and protein expression data. Some of these pathways were identified by Olah *et al.* [10] as being important in microglia that mediate remyelination in the cuprizone model. In what follows, we highlight some of the features of these mechanistic networks emphasizing activities of gene products that we have demonstrated are expressed at the protein, as well as transcript, level by DUOC-01. We note at the outset that most of the gene products we discuss have multiple biological effects. Depending on the nature of an injury and the timing of when the gene products are mobilized, some of these products could potentially contribute to harmful inflammatory or pathological effects as well as reparative outcomes. Having acknowledged that, we will focus solely on potential regenerative effects because we have demonstrated that DUOC-01 treatment enhanced remyelination in the cuprizone model [9], showed no toxicity in the preclinical safety studies required to obtain clearance to initiate clinical trial NCT02254863 and, as of December 2016, caused no apparent adverse reactions in that ongoing trial.

On the basis of our finding that DUOC-01 overexpressed transcripts for several matrix modifying proteases and secreted large amounts of MMP12 protein, we suggested [9] that DUOC-01 could regulate remyelination and repair by modifying the extracellular matrix. Activities of the brain matrix and matrix proteases in control of development and repair have been reviewed [11,12]. Figure 3 shows that transcripts for matrix modifying proteases MMP7, MMP12, CPE, MMP9, CPSK, CTSL, CTSB and NLN are abundant in DUOC-01. MMP7, MMP12 and CPE are not expressed by CD14$^+$ monocytes; conversely CD14$^+$ monocytes, but not DUOC-01, express MMP25, demonstrating specific regulation of this family of molecules. MMP2, MMP7, MMP9 and MMP12 proteins are released into the medium in

substantial amounts during manufacture of DUOC-01. Significantly, DUOC-01, but not $CD14^+$ monocytes, also over-expresses transcripts encoding A2M and TIMP3, the primary proteins that regulate the activity of matrix proteases. Furthermore, transcripts for matrix proteins constitute another of gene products that are expressed by DUOC-01. COL6A1 and COL6A2 transcripts are more than 1000-fold more abundant in DUOC-01 than in $CD14^+$ monocytes, and SPP1, FN1 and SPARC are also highly over-expressed. Again, regulation of these matrix proteins appears to be specifically coordinated: $CD14^+$ monocytes do not express the COL6A1, COL6A2, A2M or TIMP3 but do express THBS1 transcripts; DUOC-01 expresses the two collagens and protease inhibitors but not THBS1. Metalloproteases, including MMP9 [13,14] and MMP12 [15] that are secreted in abundance by DUOC-01, can affect synaptic plasticity and neural sprouting through effects on the matrix. Thus, these results suggest that several pathways coordinately regulated in DUOC-01 during manufacturing can degrade the extracellular matrix in a damaged brain and rebuild it by secreting new proteins. Consistent with this idea, transcripts for HS3STI1, a gene that encodes an enzyme that modifies heparin glycosaminoglycans; genes for signaling adherence receptors genes, VACAM1, ITGA6, ITGB8, NRP1, NRP2 and NEDD9 that regulate cell migration and interactions with the extracellular matrix; and complement component C1Qc that participates in synaptic remodeling [16] are abundant in DUOC-01, but we have not yet confirmed the level of the corresponding proteins.

Another new result reported here is that DUOC-01 also secretes many CC- and CXC-type chemokines. Transcripts for chemokines CCL22 and CCL13 were uniquely expressed by DUOC-01; CCL2 and CCL4 transcripts were over-expressed relative to $CD14^+$ monocytes; and CXCL8 transcripts were somewhat less strongly expressed in DUOC-01. CXCL12 was not differentially expressed. Immunoassay of culture supernatants showed that proteins corresponding to all these chemokines measured in the custom qPCR array as well as CCL8, CCL13, CCL15, CCL20 and CCL23 proteins accumulated at significant concentrations in culture supernatants during manufacturing. The relative amounts of each cytokine protein accumulating in the supernatants did not always reflect the relative abundance of transcripts. Collectively these secreted cytokines can potentially modulate the activity of many cell types bearing CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CXCR1, CXCR2, CXCR4 or CXCR7 receptors that are involved in brain inflammatory and repair reactions. Although expression of all these chemokines was up-regulated in DUOC-01, transcripts for chemokine receptors CCR2 and CX3CR1 could not be detected. In contrast, $CD14^+$ monocytes expressed transcripts for these receptors. Both DUOC-01 and $CD14^+$ monocytes express CXCR4.

Some of the chemokines secreted by DUOC-01 enhance remyelination. Thus, CXCL12 (SPDF) protein controls migration of neural and oligodendrocyte progenitors bearing CXCR4 receptors to demyelinated areas and promotes myelination in animals and culture systems [17–21]. We previously showed [9] that treatment with DUOC-01 drives proliferation and differentiation of oligodendrocyte progenitors and attributed this to secretion the activity of PDGFα, IGF1 and KITL proteins secreted by the cell product; CXCL12 secretion could augment this activity. Chemokines also attract microglia to lesions during remyelination processes. In one recent animal study [22], for example, astrocyte-generated IL-6 reduced local chemokine secretion, which, in turn, reduced microglial infiltration, debris removal through the microglial TREM2 receptor and finally remyelination. The chemokines involved included CCL4, major secretory product of DUOC-01. We previously showed, and confirm here, that IL-6 is a major, regulated secretory product of DUOC-01 [8]; this DUOC-01 could supplement astrocyte secretion of this cytokine. Finally, chemokine CXCL8 (IL8) is strongly angiogenic. VEGFA transcripts were also abundant in DUOC-01, suggesting that DUOC-01 can promote formation of new blood vessels following brain injury.

DUOC-01 could also modulate brain remyelination by TREM2-mediated phagocytic removal of dead cells and myelin debris from an injury site followed by signaling to glial cells [9]. Evidence for the importance of the microglial activities including TREM2-mediated phagocytosis in brain repair in the cuprizone model [23] and in human neurodegenerative conditions [24–27] continues to accumulate. DUOC-01 cells are highly phagocytic in culture [8], and we confirm [9] here that TREM1 is down-regulated and TREM2 up-regulated during DUOC-01 manufacturing. Furthermore, we found that DUOC-01 cells also express an array of transcripts related to lipid uptake, signaling and metabolism—activities associated with handling of myelin debris. These transcripts include lipid binding molecules APOE, APOC1 and COLEC112; lipid uptake receptors LRP5, LRP11 and LRP12; and lipid degrading LPL. Recent work has shown that APOE and other lipid particles are taken up by brain microglia through TREM2 and that other potentially neurotoxic molecules such as amyloid components can also be cleared by this mechanism [28]. Additionally, with regard to modulation of myelination through lipid mediated reactions, transcripts encoding several enzymes responsible for synthesizing

and converting prostaglandins from lipids (PTGDS, HPGDS, PTGES, PTGFRN, PTGR1 and PTGR2) are over-expressed in DUOC-01. Thus, DUOC-01 appears to be activated to handle lipids from degraded myelin in several ways that modulate the brain repair.

The qPCR data imply other potential mechanisms for enhancing brain repair. Among these, the array data also suggest that DUOC-01 has characteristics of reparative, as opposed to pro-inflammatory, macrophage. We confirm [8] here in additional manufacturing batches that DUOC-01 secretes substantial amounts of the immunosuppressive cytokine IL-10. The expression of NRP1, NRP2 transcripts by DUOC-01 is interesting in this regard as human monocytes that differentiate in environments leading to an M2 type polarization express these receptors [29]. We also detected high expression of transcripts for three secreted proteins, CTH, GPX3 and, especially, SEPP1, that can enhance brain repair by regulating local redox potential.

From a manufacturing perspective, the changes in gene expression described here have allowed us to assign specific gene expression signatures that characterize CB CD14$^+$ monocytes, the cells in CB that give rise to DUOC-01 during manufacturing, and the cells in the final cell product. Because the all the transcripts we studied appear to be regulated coordinately during the first 2 weeks of the manufacturing process, because we included redundant probes for some repair pathways in our custom array and because we could detect accumulation of 16 secreted proteins in the supernatants of cultures by day 14, we anticipate that smaller qPCR arrays along with assays of protein accumulation in the culture medium will become useful for in process testing and as potency assays to release product based on biological activity. Together these results suggest that it may be possible to shorten the manufacturing process provided functional characteristics are fully developed. However, as already noted, the relationship between the gene products measured for manufacturing purposes and the mechanism of action of DUOC-01 remains to be firmly established. We selected gene products that show large changes in expression during manufacturing for analysis. Genes that show less extreme or even no changes in transcriptional regulation could contribute to the activity of DUOC-01 in enhancing remyelination.

Finally, these results further support the hypothesis that cells in DUOC-01 arise from CD14$^+$ monocytes present in cultured CB MNC [9]. Thus, CB MNC and CB CD14$^+$ monocytes respond to the procedures used to manufacture DUOC-01 regulate transcript abundance in similar ways and accumulate similar amounts of proteins in the culture medium with very similar time courses. Whole transcriptome analysis by microarrays confirmed that these cell products were highly similar. We conclude that manufacturing cell products from CB MNC, the method used to make the DUOC-01 cell product that is currently in the clinic, or from CB CD14$^+$ monocytes results cell products that are highly similar with regard to all the analytical metrics we have used. We continue to be interested in determining whether cell products derived from CD14$^+$ cells and DUOC-01 have similar activity in remyelination assays because such work may provide important mechanistic insights. However, given the additional complexity, expense, lower yield and higher risk of manufacturing from the purified CD14$^+$ monocytes, we are only pursing clinical development of the DUOC-01 product made from CB MNCs at this time.

In one respect, the high degree of similarity between cell products derived from MNCs and from purified CD14$^+$ monocytes is surprising. The DUOC-01 population that emerges from cultured CB MNCs, unlike the cell product that arises from purified CD14$^+$ monocytes, is exposed to many CB erythrocytes and dead and dying leucocytes. High concentrations of mature, non-nucleated erythrocytes in culture adversely influences the yield of DUOC-01 from manufacturing [30]. During manufacturing, many dead CB cells are phagocytosed by the adherent cells that become part of the cell product [8]. Human macrophage can be activated by different agents into many different activation states in which the cells express very different gene products [31,32]. Phagocytosis of dead cells induces a unique macrophage activation state [33]. Erythrocyte-derived heme can alter macrophage activation [34]. Nevertheless, DUOC-01 derived from MNC and cell products derived from CD14$^+$ monocytes are similar. CB monocytes and macrophage have been reported by many [35–41], but not all [42], workers to differ from adult peripheral blood monocytes with regard to their ability to be activated by toll-like receptor agonists and other agents. Fetal and adult monocytes also differ in response to several inflammatory stimuli [43]. Of particular interest, human CB-derived macrophage do not become apoptotic after phagocytosis of bacteria [44–48] or apoptotic leucocytes [49] as readily as macrophage-derived from adult monocytes, and phagocytosis by the CB and adult monocyte derived macrophage results in elaboration of different cytokines [44,45,49,50]. CB monocytes also differ from adult monocytes in their ability to phagocytose proteins that may be related to Alzheimer disease onset [51]. These unique attributes of CB CD14$^+$ monocytes are likely to determine the biological activities of the cell products derived from them.

In summary, then, the biological activities of DUOC-01 arise from rapid changes in expression of

multiple genes in many pathways that promote remyelination. Many of these pathways are similar to pathways activated in microglia that regulate brain repair mechanisms. Expression of these activities is a response of CB $CD14^+$ monocytes to the other components of CB MNCs, the culture conditions and the processes used to manufacture the cell product. The physiological attributes of CB $CD14^+$ monocytes that position them to respond to manufacturing conditions by developing into cells with potentially important therapeutic activities in treating demyelinating diseases remain to be determined.

Acknowledgments

The authors are grateful to the staff at the Carolinas Cord Blood Bank for providing cord blood units for the experiments, to the staff of the CT2 GMP Cell Manufacturing Facility for help in DUOC-01 cell preparation and to Zhengzheng Wei at the Duke Institute for Genomic Sciences Microarray Core Facility for performing and helping analyze microarray data. This work was supported by the Marcus Foundation.

Disclosure of interest: The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

References

[1] Davies CL, Miron VE. Distinct origins, gene expression and function of microglia and monocyte-derived macrophages in CNS myelin injury and regeneration. Clin Immunol 2016;doi:10.1016/j.clim.2016.06.016.
[2] Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. Brain Res 2015;1619:1–11.
[3] Jin X, Yamashita T. Microglia in central nervous system repair after injury. J Biochem 2016;159:491–6.
[4] Lampron A, Pimentel-Coelho PM, Rivest S. Migration of bone marrow-derived cells into the CNS in models of neurodegeneration. J Comp Neurol 2013;521(17):3863–76.
[5] Miron VE, Franklin RJ. Macrophages and CNS remyelination. J Neurochem 2014;130:165–71.
[6] Shechter R, Schwartz M. Harnessing monocyte-derived macrophages to control central nervous system pathologies: no longer "if" but "how. J Pathol 2013;229:332–46.
[7] Rawji KS, Mishra MK, Yong VW. Regenerative capacity of macrophages for remyelination. Front Cell Dev Biol 2016;4:47.
[8] Kurtzberg J, Buntz S, Gentry T, Noeldner P, Ozamiz A, Rusche B, et al. Reprint of: preclinical characterization of DUOC-01, a cell therapy product derived from banked umbilical cord blood for use as an adjuvant to umbilical cord blood transplantation for treatment of inherited metabolic diseases. Cytotherapy 2015;17:1314–26.
[9] Saha A, Buntz S, Scotland P, Xu L, Noeldner P, Patel S, et al. A cord blood monocyte-derived cell therapy product accelerates brain remyelination. JCI Insight 2016;1:e86667.
[10] Olah M, Amor S, Brouwer N, Vinet J, Eggen B, Biber K, et al. Identification of a microglia phenotype supportive of remyelination. Glia 2012;60:306–21.
[11] Ethell IM, Ethell DW. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J Neurosci Res 2007;85:2813–23.
[12] Luo J. The role of matrix metalloproteinases in the morphogenesis of the cerebellar cortex. Cerebellum 2005;4:239–45.
[13] Dziembowska M, Wlodarczyk J. MMP9: a novel function in synaptic plasticity. Int J Biochem Cell Biol 2012;44:709–13.
[14] Kelly EA, Russo AS, Jackson CD, Lamantia CE, Majewska AK. Proteolytic regulation of synaptic plasticity in the mouse primary visual cortex: analysis of matrix metalloproteinase 9 deficient mice. Front Cell Neurosci 2015;9:369.
[15] Elisia I, Nakamura H, Lam V, Hofs E, Cederberg R, Cait J, et al. DMSO represses inflammatory cytokine production from human blood cells and reduces autoimmune arthritis. PLoS ONE 2016;11:e0152538.
[16] Bialas AR, Stevens B. TGF-beta signaling regulates neuronal C1q expression and developmental synaptic refinement. Nat Neurosci 2013;16:1773–82.
[17] Dziembowska M, Tham TN, Lau P, Vitry S, Lazarini F, Dubois-Dalcq M. A role for CXCR4 signaling in survival and migration of neural and oligodendrocyte precursors. Glia 2005;50:258–69.
[18] Carbajal KS, Schaumburg C, Strieter R, Kane J, Lane TE. Migration of engrafted neural stem cells is mediated by CXCL12 signaling through CXCR4 in a viral model of multiple sclerosis. Proc Natl Acad Sci USA 2010;107:11068–73.
[19] Banisadr G, Frederick TJ, Freitag C, Ren D, Jung H, Miller SD, et al. The role of CXCR4 signaling in the migration of transplanted oligodendrocyte progenitors into the cerebral white matter. Neurobiol Dis 2011;44:19–27.
[20] Li Y, Tang G, Liu Y, He X, Huang J, Lin X, et al. CXCL12 Gene Therapy Ameliorates Ischemia-Induced White Matter Injury in Mouse Brain. Stem Cells Transl Med 2015;4:1122–30.
[21] Zilkha-Falb R, Kaushansky N, Kawakami N, Ben-Nun A. Post-CNS-inflammation expression of CXCL12 promotes the endogenous myelin/neuronal repair capacity following spontaneous recovery from multiple sclerosis-like disease. J Neuroinflammation 2016;13:7.
[22] Petkovic F, Campbell IL, Gonzalez B, Castellano B. Astrocyte-targeted production of interleukin-6 reduces astroglial and microglial activation in the cuprizone demyelination model: implications for myelin clearance and oligodendrocyte maturation. Glia 2016;64:2104–19.
[23] Cantoni C, Bollman B, Licastro D, Xie M, Mikesell R, Schmidt R, et al. TREM2 regulates microglial cell activation in response to demyelination in vivo. Acta Neuropathol 2015;129:429–47.
[24] Walter J. The triggering receptor expressed on myeloid cells 2: a molecular link of neuroinflammation and neurodegenerative diseases. J Biol Chem 2016;291:4334–41.
[25] Ulrich JD, Holtzman DM. TREM2 function in alzheimer's disease and neurodegeneration. ACS Chem Neurosci 2016;7:420–7.
[26] Han J, Wang M, Ren M, Lou H. Contributions of triggering-receptor-expressed-on-myeloid-cells-2 to neurological diseases. Int J Neurosci 2016;1–17.
[27] Andreasson KI, Bachstetter AD, Colonna M, Ginhoux F, Holmes C, Lamb B, et al. Targeting innate immunity for neurodegenerative disorders of the central nervous system. J Neurochem 2016;138:653–93.
[28] Yeh FL, Wang Y, Tom I, Gonzalez LC, Sheng M. TREM2 binds to apolipoproteins, including APOE and CLU/APOJ, and thereby facilitates uptake of amyloid-beta by microglia. Neuron 2016;91:328–40.

[29] Ji JD, Park-Min KH, Ivashkiv LB. Expression and function of semaphorin 3A and its receptors in human monocyte-derived macrophages. Hum Immunol 2009;70:211–17.
[30] Tracy ET, Zhang CY, Gentry T, Shoulars KW, Kurtzberg J. Isolation and expansion of oligodendrocyte progenitor cells from cryopreserved human umbilical cord blood. Cytotherapy 2011;13:722–9.
[31] Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity 2014;40:274–88.
[32] Ginhoux F, Schultze JL, Murray PJ, Ochando J, Biswas SK. New insights into the multidimensional concept of macrophage ontogeny, activation and function. Nat Immunol 2015;17:34–40.
[33] Ariel A, Serhan CN. New lives given by cell death: macrophage differentiation following their encounter with apoptotic leukocytes during the resolution of inflammation. Front Immunol 2012;3:4.
[34] Naito Y, Takagi T, Higashimura Y. Heme oxygenase-1 and anti-inflammatory M2 macrophages. Arch Biochem Biophys 2014;564:83–8.
[35] Pedraza-Sanchez S, Hise AG, Ramachandra L, Arechavaleta-Velasco F, King CL. Reduced frequency of a $CD14^{+}$ $CD16^{+}$ monocyte subset with high toll-like receptor 4 expression in cord blood compared to adult blood contributes to lipopolysaccharide hyporesponsiveness in newborns. Clin Vaccine Immunol 2013;20:962–71.
[36] Perez A, Bellon JM, Gurbindo MD, Munoz-Fernandez MA. Impairment of stimulation ability of very-preterm neonatal monocytes in response to lipopolysaccharide. Hum Immunol 2010;71:151–7.
[37] Belderbos ME, van Bleek GM, Levy O, Blanken MO, Houben ML, Schuijff L, et al. Skewed pattern of Toll-like receptor 4-mediated cytokine production in human neonatal blood: low LPS-induced IL-12p70 and high IL-10 persist throughout the first month of life. Clin Immunol 2009;133:228–37.
[38] Morgado JM, Pratas R, Laranjeira P, Henriques A, Crespo I, Regateiro F, et al. The phenotypical and functional characteristics of cord blood monocytes and $CD14(^{-}/low)/CD16(^{+})$ dendritic cells can be relevant to the development of cellular immune responses after transplantation. Transpl Immunol 2008;19:55–63.
[39] Yan SR, Qing G, Byers DM, Stadnyk AW, Al-Hertani W, Bortolussi R. Role of MyD88 in diminished tumor necrosis factor alpha production by newborn mononuclear cells in response to lipopolysaccharide. Infect Immun 2004;72:1223–9.
[40] Levy O, Zarember KA, Roy RM, Cywes C, Godowski PJ, Wessels MR. Selective impairment of TLR-mediated innate immunity in human newborns: neonatal blood plasma reduces monocyte TNF-alpha induction by bacterial lipopeptides, lipopolysaccharide, and imiquimod, but preserves the response to R-848. J Immunol 2004;173:4627–34.
[41] Brichard B, Varis I, Latinne D, Deneys V, de Bruyere M, Leveugle P, et al. Intracellular cytokine profile of cord and adult blood monocytes. Bone Marrow Transplant 2001;27:1081–6.
[42] Sohlberg E, Saghafian-Hedengren S, Bremme K, Sverremark-Ekstrom E. Cord blood monocyte subsets are similar to adult and show potent peptidoglycan-stimulated cytokine responses. Immunology 2011;133:41–50.
[43] Krow-Lucal ER, Kim CC, Burt TD, McCune JM. Distinct functional programming of human fetal and adult monocytes. Blood 2014;123:1897–904.
[44] Gille C, Dreschers S, Leiber A, Lepiorz F, Krusch M, Grosse-Opphoff J, et al. The CD95/CD95L pathway is involved in phagocytosis-induced cell death of monocytes and may account for sustained inflammation in neonates. Pediatr Res 2013;73:402–8.
[45] Gille C, Dreschers S, Spring B, Tarnok A, Bocsi J, Poets CF, et al. Differential modulation of cord blood and peripheral blood monocytes by intravenous immunoglobulin. Cytometry B Clin Cytom 2012;82:26–34.
[46] Gille C, Leiber A, Mundle I, Spring B, Abele H, Spellerberg B, et al. Phagocytosis and postphagocytic reaction of cord blood and adult blood monocyte after infection with green fluorescent protein-labeled Escherichia coli and group B Streptococci. Cytometry B Clin Cytom 2009;76:271–84.
[47] Gille C, Leiber A, Spring B, Kempf VA, Loeffler J, Poets CF, et al. Diminished phagocytosis-induced cell death (PICD) in neonatal monocytes upon infection with Escherichia coli. Pediatr Res 2008;63:33–8.
[48] Berner R, Welter P, Brandis M. Cytokine expression of cord and adult blood mononuclear cells in response to Streptococcus agalactiae. Pediatr Res 2002;51:304–9.
[49] Gille C, Steffen F, Lauber K, Keppeler H, Leiber A, Spring B, et al. Clearance of apoptotic neutrophils is diminished in cord blood monocytes and does not lead to reduced IL-8 production. Pediatr Res 2009;66:507–12.
[50] Gille C, Spring B, Tewes LJ, Loffler J, Dannecker GE, Hoffmann MK, et al. Diminished response to interleukin-10 and reduced antibody-dependent cellular cytotoxicity of cord blood monocyte-derived macrophages. Pediatr Res 2006;60:152–7.
[51] Darlington D, Deng J, Giunta B, Hou H, Sanberg CD, Kuzmin-Nichols N, et al. Multiple low-dose infusions of human umbilical cord blood cells improve cognitive impairments and reduce amyloid-beta-associated neuropathology in Alzheimer mice. Stem Cells Dev 2013;22:412–21.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jcyt.2017.03.004.