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Evaluation of gene expression profiles of immature dendritic cells prepared from peripheral blood mononuclear cells

Jeong Won Shin^{1,2}, Ping Jin¹, Yong Fan¹, Stefanie Slezak¹, Virginia David-Ocampo¹, Hanh M. Khuu¹, Elizabeth J. Read^{1,3}, Ena Wang¹, Francesco M. Marincola¹, and David F. Stroncek¹

¹Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, Maryland, USA

²Department of Laboratory Medicine, Soonchunhyang University Hospital, Seoul, Korea

³Blood Systems Research Institute, San Francisco, California, USA

Abstract

Objective—Dendritic cells (DCs) generated ex vivo from peripheral blood monocytes or mobilized CD34+ cells and intended for clinical immunotherapy are typically characterized by morphologic, phenotypic, and functional assays. Assay results are highly dependent on conditions used to prepare the cells, so there is no standard assay battery for clinical DC products. This study evaluated gene expression profiling for characterization of immature DCs prepared from monocytes that had been elutriated from normal donor peripheral blood mononuclear cells (PBMNCs) immediately after collection or after storage at 4°C for 48 hours.

Methods—RNA was isolated from fresh and 48-hour-stored PBMNCs, elutriated monocytes, elutriated lymphocytes and immature DCs from 5 healthy subjects and was analyzed using a cDNA gene expression microarray with 17,500 genes.

Results—Unsupervised hierarchical clustering separated the 40 products into 4 groups: one with all 10 immature DCs, one with all 10 elutriated lymphocytes, one with 7 PBMNCs, and one with 10 elutriated monocytes and 3 PBMNCs. However, within each of the 4 groups, fresh and stored products, or products derived from fresh or stored products, clustered together. Comparison of genes differentially expressed by fresh vs stored products (paired t-tests, $p < 0.005$) found 273 genes that differed between fresh and stored PBMNCs, 429 between lymphocytes elutriated from fresh vs stored PBMNCs, 711 between monocytes elutriated from fresh vs stored PBMNCs, and 3 between immature DCs prepared from monocytes elutriated from fresh vs stored PBMNCs.

Conclusions—This study demonstrates the potential utility of gene expression profiling for characterization of cell therapy products.

INTRODUCTION

Dendritic cell (DC) tumor vaccines are a growing area of cancer immunotherapy. DCs are typically generated by culture of peripheral blood monocytes or CD34+ hematopoietic progenitor cells.^{1–7} A variety of methods have been published on preparation of immature DCs, the most common being culture in IL-4 and GM-CSF.^{4–6} An even greater variety of

Address all correspondence to: David Stroncek, MD, Department of Transfusion Medicine, National Institutes of Health, Warren G. Magnuson Clinical Center, Building 10, Room 1C711, 10 Center Drive MSC-1184, Bethesda, MD, USA 20892-1184, Phone: (301) 496-8335, Fax: (301) 402-1360 Email: E-mail: dstroncek@cc.nih.gov.

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methodologic variations has been published for generation of mature from immature DCs.^{1–3,8} Characterization of the resulting DCs by morphologic, phenotypic, and functional assays plays an important role in developmental research and release testing of DC products, but assay results are dependent on details of the DC manufacturing process.⁸

Gene expression profiling is a global approach to characterization of cells that shows what genes are up- and down-regulated in relationship to a control cell population. Gene expression profiling has been used to predict the confluence of human embryonic kidney 293 cells used to manufacture adenovirus vectors and vaccines⁹ and the differentiation of status of human embryonic stem cells and embryoid bodies.^{10,11} Gene expression profiles also differ among immature and mature DCs and DCs produced from CD34+ cells or peripheral blood mononuclear cells (PBMNCs).^{3,12–14}

The production of clinical cellular therapies often involves multiple centers. Cells such as PBMNCs are collected from a donor or patient at one center and then shipped to a specialized cellular therapy laboratory where they are processed and cells such as DCs are produced. When processing is complete, the product is shipped to the site where it is administered to a specific patient. The centralized cell processing laboratory may be hundreds or thousands of miles from the collection center and it may take up 48-hours after the collection is complete for the starting cellular material to reach the cell processing laboratory.

The purpose of this study was to evaluate the use of gene expression profiling on immature DCs prepared from peripheral blood monocytes that had been elutriated from PBMNCs. In addition to comparing gene expression in immature DC products with the elutriated monocytes and PBMNCs from which they were prepared, as well as the lymphocyte by-product from PBMNC elutriation, we also evaluated the effect of 48-hour storage of PBMNCs on gene expression profiles.

MATERIAL AND METHODS

PBMC collection and sample preparation

Human subjects were 5 healthy adult volunteers after signing informed consent to a protocol that had approved by a NIH Institutional Review Board (IRB). Each subject had PBMNCs collected using a Cobe Spectra leukapheresis system (Gambro BCT, Lakewood, CO) or a CS3000 plus blood cell separator (Baxter Healthcare Corp., Fenwal Division, Deerfield, IL). The subjects were not treated with cytokines or growth factors prior to the collection procedure. After leukapheresis, the PBMNC concentrates were divided equally into two portions. One half was elutriated on the same day of collection to produce enriched monocyte and lymphocyte fractions and the other half was processed after being stored at 4°C for 48 hours in a bag (Transfer Pack Container, Baxter Healthcare Corp., Fenwal Division). Prior to elutriation a sample of the PBMNC product was removed processed to remove contaminating red cells and granulocytes by Ficoll-Hypaque (Pharmacia Biotechnology, Uppsala, Sweden) density gradient separation, addition of RLT buffer (Qiagen, Valencia, CA) with 2-Mercaptoethanol, immediate freezing, and storage at –80°C until analysis.

Elutriation

Counterflow centrifugal elutriation was performed using the Elutra® (Gambro BCT, Lakewood, CO) with the automatic mode according to the manufacturer's recommendations. Among the five elutriation fractions, the lymphocyte fraction was collected at a flow rate of 120mL/min during centrifugation and the monocyte fraction was obtained at 124 mL/min with the rotor off.

Immature DC preparation

Immature DCs were generated from fresh and stored elutriated monocytes from each donor as previously described.^{7,8} Briefly, 1.5×10^6 cells/mL were placed in a T25 culture flask (Nalge Nunc International, Rochester, NY) with RPMI 1640 (Cambrex, Walkersville, MD) supplemented with 10% human AB serum, 10 µg/mL gentamicin (Cambrex, Walkersville, MD), 2000 IU/mL human IL-4 (Schering-Plough, Kenilworth, NJ) and 2000 IU/mL GM-CSF (Amgen, Thousand Oaks, CA). On Day 2, an additional 2000 IU/mL IL-4 and GM-CSF were added. Immature DCs were harvested on Day 3 using gentle agitation and analyzed.

RNA preparation, amplification and labeling

Total RNA was extracted from PBMNCs, elutriated monocytes, elutriated lymphocytes, and immature DCs using the RNeasy minikit (Qiagen, Valencia, CA) and 0.01–5 µg of them were amplified into anti-sense RNA (aRNA). Also, total RNA from PBMNCs pooled from six normal donors was extracted and amplified into aRNA to serve as the reference.¹⁵ These six normal donors were different from the six PBMNC donors. Test and reference RNAs were labeled with Cy5 (red) and Cy3 (green) dyes, respectively, and co-hybridized to the in-house prepared 17.5K cDNA (UniGene cluster) microarray which were printed with a configuration of $32 \times 24 \times 23$.¹⁵ Clones used for printing included a combination of the Research Genetics RG_HsKG_031901 8k clone set and 9,000 clones selected from the RG_Hs_seq_ver_070700 40k clone set. The 17,500 spots included 12,072 uniquely named genes, 875 duplicated genes and about 4,000 expression sequence tags. The complete list of genes included in the Hs-CCDTV-17.5k-1px printing is available at the web site http://nciarray.nci.nih.gov/gal_files/index.shtml.

Data processing and statistical analyses

The raw data set was filtered according to standard procedure to exclude spots with minimum intensity that arbitrarily set to an intensity parameter of 300 in both fluorescence channels. If the fluorescence intensity of one channel was greater than and that of the other but was below 300, the fluorescence of the low intensity channel was arbitrarily set to 300. Spots with diameters <25µm and spots flagged by the software as inadequate were also excluded from the analyses. Then, the filtered data were normalized using Lowess Smoother and retrieved by the BRB ArrayTool (<http://linus.nci.nih.gov/BRB-ArrayTools.html>) developed at the National Cancer Institute (NCI), Biometric Research Branch, Division of Cancer Treatment and Diagnosis. We selected genes that were expressed in over 80% of samples for analyses. According to this, 8,661 genes were selected and we conducted hierarchical cluster analysis on those genes using Cluster and TreeView software^{15,16}. For annotation of genes and functional pathways, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) 2007 software¹⁷ and GeneCards website (<http://www.genecards.org/index.shtml>).

RESULTS

Donors and Products

Healthy volunteer subjects were aged 29 to 68 years, with 4 males and 1 female. Two of the subjects were Caucasian, 2 African-American, and 1 Hispanic (Table 1). For PBMNC products, a mean of 10.3 ± 1.1 L of blood was processed to obtain products with a volume of 137 ± 8 mL, and the total white blood cell content of the PBMNC products was $10.0 \pm 3.0 \times 10^9$ that were $87.8 \pm 12.2\%$ mononuclear cells (monocytes + lymphocytes) (Table 1). The elutriated monocyte fractions prepared from the fresh and 48-hour-stored PBMNCs contained $84 \pm 8\%$ (range 77% to 96%) and $75 \pm 13\%$ (range 71% to 94%) monocytes, respectively. The elutriated

lymphocyte fractions prepared from fresh and stored PBMNCs contained $98 \pm 1\%$ (range 97% to 99%) and $97 \pm 2\%$ (95% to 97%) lymphocytes, respectively.

Gene expression profiling of all products by unsupervised hierarchical clustering

Hierarchical clustering was applied to the genes expressed by the 40 products: fresh and stored PBMNCs and elutriated lymphocytes, elutriated monocytes, and immature DCs prepared from the fresh and 48-hour-stored PBMNCs. After filtering, unsupervised hierarchical clustering of the remaining 8,661 genes separated the products into 4 different clusters: one made up of all 10 elutriated lymphocyte products, one with 7 PBMNC products, one with all 10 elutriated monocyte and 3 PBMNC products and another with all 10 immature DC products (Figure 1).

While hierarchical clustering did not separate the fresh and stored PBMNCs or products derived from fresh or stored PBMNCs, a comparison of fresh and 48-hour-stored products revealed some differences. When fresh and 48-hour-stored PBMNCs were compared using paired t-test ($p < 0.005$) among the 8,661 genes analyzed the expression of 273 genes differed among PBMNCs and the expression of 429, 711, and 3 differed between elutriated lymphocytes, elutriated monocytes, and immature DCs prepared from fresh and 48-hour-stored PBMNCs (Table 2). This shows that while there are some differences between fresh and stored PBMNCs and elutriated monocytes and lymphocytes prepared from fresh and stored PBMNCs, there was little difference between immature DCs prepared from fresh and stored PBMNCs.

In general, within each group the fresh and 48-hour-stored samples from the same donor clustered together (Figure 1). These results suggested that differences among donors were more significant than differences due to 48 hours of storage. When the number of genes in each type of product that differed among the 5 donors was analyzed a total of 336, 455, 1003 and 73 genes differed significantly (F-test, $p < 0.005$) among the donors in PBMNCs, elutriated lymphocytes, elutriated monocytes, and immature DCs respectively, which was similar to the number of genes whose expression changed with storage of the respective products.

Analysis of genes differentially expressed between products prepared from fresh and stored PBMNCs

Supervised hierarchical clustering was performed with genes whose expression differed between fresh and 48-hour-stored PBMNCs and elutriated lymphocytes and elutriated monocytes prepared from fresh and stored PBMNCs (Figure 2, Figure 3, and Figure 4). The number of genes with expression differences between immature DC prepared from fresh vs those prepared from stored PBMNCs was insufficient for analysis.

Among PBMNCs, elutriated lymphocyte, and elutriated monocyte products the types of genes whose expression changed with storage were similar. The greatest numbers of genes whose expression increased with storage were associated with cytokines/chemokines and their receptors, apoptosis, stress response, and immune function. The specific genes involved with cytokines/chemokines and their receptors whose expression increased included CCL2, CCL4, CCL7, CCL20, CCL23, CXCL1, IL1, IL8, IL18, IL23, IL6R, INFG, TNF, SF1; specific apoptosis-related genes included PHLDA, GAS1; stress response genes included TGFB, APR, oncogenes including myc, fos and junD; and immune function related genes included CSF1, GATA3, ICOS, ITK. The expression of some genes encoding proteins that involve cell transcription and membrane transport were also increased, for example, SLCs, HISTs. On the other hand, genes related to cell metabolism (PIKs, PLA2G6, PTPRC, STK, CDKN1C), anti-apoptosis (SOCS3, API5, NGFB), immunoglobulins (MGC27165, IGHG1, IGLL1, IGL) and some cytokines/chemokines and their receptors (CCL3, CXCL10, CX3CR1, CCR2, IL16, IL18R1, IL24) showed decreased expression after storage.

The genes whose expression changed during storage belonged to several functional pathways. The pathways that included more than 5 genes that were up-regulated during storage were similar for monocytes and lymphocytes (Table 3 and Table 4). In general, these pathways involved cell activation, signaling, and response to stress. Genes in cytokine-cytokine receptor interaction pathway, MAPK signaling pathway, T cell receptor signaling pathway, and Toll-like receptor signaling pathway were up-regulated in both monocytes and lymphocytes. Genes in the NK cell mediated cytotoxicity were only up-regulated in monocytes, whereas, insulin-signaling, Fc epsilon RI signaling, glycan structures-biosynthesis, focal adhesion and MAPK signaling pathways were down-regulated with storage in monocytes. However, in lymphocytes no pathway had more than 5 genes down-regulated.

Genes differentially expressed by immature DCs

To confirm that the cultured elutriated monocytes had differentiated into immature DCs, we identified genes whose expression differed more than 3-fold between the immature DC and elutriated monocytes (paired t-tests, $p < 0.005$). The expression of 879 genes differed between the two groups. A review of these genes revealed that 285 had previously been found to be differentially expressed during DC maturation (Appendix).^{1,3,13,14,18-23} Of these 285 genes, 115 genes were up-regulated in immature DCs compared to elutriated monocytes and 170 were down-regulated.

The 115 genes that were up-regulated in immature DCs included cell surface proteins MRC1, FCER2(CD23), CLDN23, CD9, CD63, LRPAP1, LAMP1, CD81, ITGAX, MHC Class II, metabolism/enzyme related proteins CYP27A1, FAIM, FLJ10116, PLA2G7, CHST11, LIPA, MAOA, FABP, ACP5, CDK4, nuclear proteins ZNF204, KIFC3, RAB38, ADORA2B, PALLD, PPARG, RRAGD, CREB3L4, IFI30, RAD1 and secretory proteins A2M, VEGFB, LGALS3, CSF1, C1QB, NMB. In addition, gene expression of signal transduction/growth control related proteins INPP5F, FNDC3B, DYRK2, PDGFB, TRAF3 and chemokine/cytokines such as CCL18, CCRL2, CCR5 and IL1R1 were also increased in immature DCs.

The 170 genes that were down-regulated in immature DCs included nuclear proteins, FOSB, KLF9, NFKBIZ, HNRPU, MXD1, RGS1, AIF1, GATA2, RNF138, JUNB, metabolism/enzyme related proteins, NR4A2, VNN2, ABCA1, S100AB, MCL1, LTF, SERPINB2, USP34, JAK3, HSPA8, Signal transduction/growth control related proteins, KCNG1, CDKN1C, GBP2, DUSP6, MAP3K8, KEAP1, CAMK1, AREG, EMR1, NFKBIA, cell surface proteins, CD62L, CD97, CD64, CD44, MCH Class I, TLR, CD24, ICAM1 (CD54), ITGA4 (CD49D), PECAM1 (CD31), CSF1R (CD115), CD163. Chemokine/cytokines and their receptors, like IL8, IL15, CXCL1, CCL4, CX3CR1, IL13RA1, TNF, IL6, IL10RA and TGFBR3 were also down-regulated in immature DCs (Appendix).

DISCUSSION

We found that 48 hours of storage of PBMNCs at 4°C had no effect on immature DCs prepared from the stored PBMNCs and little effect on lymphocytes and monocytes separated from stored PBMNCs. The changes noted in monocytes during storage did not effect the ability of these cells to be used to produce immature DCs. In fact, there were far fewer differences among immature DCs derived from fresh and 48-hour-stored PBMNCs than between monocytes and lymphocytes prepared from either fresh and stored PBMNCs. It appears that the changes in gene expression associated with the transformation of monocytes to immature DCs are much greater than any change in monocytes that occurred as a result of storage. Although we did not specifically compare the function of immature DCs prepared from fresh and stored PBMNCs, the striking similarity in gene expression among immature DCs derived from fresh and stored PBMNCs suggests that there will be little differences in the function of these cells.

The changes in gene expression noted in stored lymphocytes were less than those noted in monocytes. In addition, the types of genes whose expression changed in lymphocytes during storage were similar to the types of genes that changed in monocytes during storage. This suggests that lymphocytes derived from 48-hour-stored PBMNCs used for clinical applications such as the treatment of relapsed leukemia or the production of tumor- or virus-specific cytotoxic lymphocytes will be as effective as those prepared from fresh PBMNCs. However, other therapies produced using lymphocytes or monocytes from stored PBMNCs should be evaluated before being used clinically.

The expression of 879 genes were differentially expressed among immature DC and monocytes, 285 of whose expression has previous been found to differ among DCs and monocytes. Among the genes whose expression was markedly increased in immature DCs were CD23, the low affinity Fc receptor of IgE, II, FCER2, the mannose receptor, MRC1, and CCL18. The expression of both CD23 and the mannose receptor were increased 58-fold and the expression of CCL18 was increased 36-fold in immature DCs. All three of these genes are important markers of immature DCs.^{1,3,13,14,18–23} The expression of all three are greater on immature DCs than mature DCs. CD23 is expressed on B cells and follicular dendritic cells, but its expression is increased on monocyte-derived DCs.^{14,24} CCL18 is one of the most abundantly produced chemokines by immature DCs.²⁵ MRC1 is important in endocytosis, pinocytosis, and phagocytosis.^{26–29} It binds bacteria, yeasts, and viruses through interactions of its carbohydrate recognition domains and high mannose structures on pathogens and is involved with presenting antigens to MHC Class II molecules. MRC1 is expressed by alveolar macrophages and lymphatic and hepatic endothelial cells and cultured immature DCs. The expression of MRC1 is often used as a marker of immature DCs.

We also found that the expression of the tetraspan CD9 and VEGF-beta were both increased 21-fold on immature DCs compared to monocytes. The tetraspan CD9 is associated with MHC II and CD38 molecules in DC plasma membrane lipid rafts and CD9 and CD38 are involved with HLA-DR signaling.^{30,31} The expression of the growth factor VEGF-alpha has been found to be increased on monocyte-derived mature and immature DCs.¹³ VEGF produced by cancer cells prevents the maturation of immature DCs.³² It is possible that VEGF has an autocrine function of maintaining DCs in the immature state.

Among the genes whose expression was increased in immature DCs compared on monocytes were cytochrome P450, subunit A, polypeptide 1 (CYP27A1) and phospholipase A2, group VII (PLA2G7) whose expression was increased 70.6-fold and 28.1-fold respectively. While the expression of cytochromes, phospholipases and related genes are increased in DCs, the expression of these specific genes have not previously been found to be increased in DCs. These two genes may be useful DC markers.

Among the genes that were markedly over expressed by monocytes compared to immature DCs were several that are important in the function of mature leukocytes. The chemotactic receptor formyl peptide receptor 1 was increased 71-fold in monocytes, the adhesion molecule CD62L was increased 40-fold, the macrophage calcium binding protein, S100A8, was increased 38-fold, CD97 12-fold and the expression of two Fc receptors, Fc gamma receptors IIa and IIIa, were both increased 9-fold.

In our study PBMNCs were stored in bags specifically designed for the storage of blood components. It is not likely that the PBMNCs stored in tubes or containers that restrict the exchange of oxygen and carbon dioxide would be maintained in the same condition as those stored in specialized blood storage bags. In fact, Baecher et al³³ compared gene expression in fresh PBMNCs with PBMNCs obtained from healthy individuals that had been stored overnight in blood collection tubes. They identified 2,034 genes that showed significant

changes in expression after overnight incubation. In contrast, our study found that the expression of only 273 PBMNC genes changed with storage.

This study demonstrates the potential for the use of gene expression profiling in cellular therapies. The analysis of DCs produced *ex vivo* for clinical application is particular problematic.^{34,35} Typically, cell function assays and flow cytometric phenotyping are used to analyze the potency, purity and maturation of DCs. Cell function assays are limited in that they are labor intensive and, generally require many hours or days to complete. In addition, the *in vivo* effectiveness of DCs is likely dependent on many cell functions so a single assay cannot assess all critical aspects of DC function. Flow cytometry is limited by the number of surface markers and cytokines that can be analyzed at one time. These limitations are compounded by the fact that the *in vivo* effectiveness of DCs may, in part, be dependent on functions or aspects of DCs that are not yet well understood or known and hence can not be assessed by cell function or flow cytometry phenotyping.

Global gene expression profiling can analyze the expression of thousands of genes using a very small number of cells. Our studies show that the gene expression profiles of immature DCs were markedly different than monocytes and lymphocytes. We suspect that the profile of immature DCs will change further as they develop into mature DCs and gene expression profiling will be useful for assessing the state of differentiation or maturation of DCs. In addition, when the results of gene expression profiling are correlated with *in vivo* bioactivity of DC therapy or the clinical outcome of DC therapy, they may provide important information concerning the role of specific genes and pathways in the effectiveness of clinical DC therapy. Comparing the gene expression profiles of DCs given to patients with good clinical outcomes with those with less favorable outcomes may lead to the identification of variables in starting cellular materials, production methods, patient conditions, and patient polymorphisms that impact the effectiveness of DC therapy.

In conclusion, we used gene expression profiling to show that there is little difference in immature DCs prepared from fresh and 48-hour-stored PBMNCs. Gene expression profiling also has the potential to be an effective tool for assessing peripheral blood leukocytes and products derived from peripheral blood leukocytes that are used in cellular therapies.

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8661 genes

Figure 1.

Gene expression profiling of fresh and 48-hour stored PBMNC products, and elutriated lymphocytes, elutriated monocytes, and immature dendritic cells (DCs) prepared from fresh and stored PBMNCs. Products from 5 healthy donors were analyzed. The 8,661 genes that remained after filtering (expressed in 80% of samples) were analyzed by unsupervised hierarchical clustering of Eisen. PBMNC products are indicated by a blue bar, elutriated lymphocytes by a red bar (Elu-Lym), elutriated monocytes by a yellow bar (Elu-Mono) and immature DCs by a green bar (DC).

Figure 2. Genes differentially expressed by 48-hour-stored PBMCs. Genes whose expression differed among fresh and 48-hour-stored PBMCs ($p < 0.005$ using paired t-tests) were analyzed by supervised hierarchical clustering of Eisen. The red box contains representative genes whose expression increased with storage and the blue box representative genes whose expression was decreased. Fresh PBMCs are indicated by the orange bar (PBMC-Fresh) and 48-hour-stored by the green bar (PBMC-48h).

Figure 3. Genes differentially expressed by 48-hour-stored elutriated lymphocyte prepared from 48-hour-stored PBMCs. Genes whose expression differed among elutriated lymphocytes prepared from fresh and 48-hour-stored PBMCs ($p < 0.005$ using paired t-tests) were analyzed by supervised hierarchical clustering of Eisen. The red box contains representative genes whose expression increased with storage and the blue box those whose expression decreased. Elutriated lymphocyte prepared from fresh PBMCs are indicated by the orange bar (Elu-Lym) and those prepared from 48-hour-stored PBMCs by the green bar (Elu-Lym48h).

Figure 4. Genes differentially expressed by elutriated monocytes prepared from 48-hour-stored PBMNCs. Genes whose expression differed among elutriated monocytes prepared from fresh and 48-hour-stored PBMNCs ($p < 0.005$ using paired t tests) were analyzed by supervised hierarchical clustering of Eisen. The red box contains representative genes whose expression increased with storage and the blue box those whose expression decreased. Elutriated monocytes prepared from fresh PBMNCs are indicated by the orange bar (Elu-Mono) and those prepared from 48-hour-stored PBMNCs by the green bar (Elu-Mono48h).

Table 1

Characteristics of donors and leukapheresis products

Donor No	Age	Sex	Race	Cell separator	Product volume	Cell counts and differentials of products		
						Total WBCs ($\times 10^9/L$)	Lymphocytes (%)	Monocytes (%)
1	48	F	African-American	Cobe Spectra	136mL	58.2	66.0	0.6
2	68	M	Caucasian	Cobe Spectra	128mL	67.5	54.9	37.5
3	32	M	African-American	Fenwal CS3000	136mL	83.4	77.5	11.4
4	29	M	Hispanic	Cobe Spectra	137mL	107.0	78.4	17.7
5	56	M	Caucasian	Cobe Spectra	150mL	50.7	66.7	28.0

Table 2

Comparison the number of genes whose expression differed between fresh and 48-hour-stored samples ($p < 0.005$, by paired t-test)

	Number of genes whose expression differed		
	Increased [*]	Decreased [†]	Total changed
PBMNCs: fresh vs. 48hr-stored	122	151	273
Elutriated lymphocytes: fresh vs. 48hr-stored	297	132	429
Elutriated monocytes: fresh vs. 48hr-stored	408	303	711
Immature DCs: fresh vs. 48hr-stored	2	1	3

* Number of genes whose expression increased in 48-hour-stored samples.

† Number of genes whose expression decreased in 48-hour-stored samples.

A total of 8,661 genes were analyzed.

Table 3

Functional pathways affected in elutriated lymphocytes prepared from PBMNCs stored for 48 hours (pathways with changes in > 5 genes)

	Pathways	Genes affected	Functions involved
Up-regulated after storage	MAPK signaling pathway	TNF, Ras, Cdc42/Rac, JunD, Tp12/Cot, MKP, JNK, c-JUN, MNK1/2, NFkB, CREB, Sap1a, c-Myc, GADD153	Apoptosis/anti-Apoptosis Stress response
	Cytokine-cytokine receptor interaction	CCL2, CCL3, CCL4, IL6R, IL23A, FLT4, IFNG, TNF	Cytokine/chemokine stimulation, Apoptosis
	T cell receptor signaling pathway	Rho/Cdc42, Ras, COT, AP1, NFkB, IkB, INFG, TNF α	Immune response
	Toll-like receptor signaling pathway	NFkB, IkB α , JNK, AP1, TNF α , MIP-1 α , MIP-1 β , CD80	Immune response, Cytokine/chemokine Stimulation, Apoptosis
	Regulation of actin cytoskeleton	Ras, ITG, Cdc42, Drf3, Arp2/3, CFN	Stress response, Cell adhesion

* No down-regulated pathway showing > 5 gene changes was detected.

Table 4

Functional pathways affected in elutriated monocytes prepared from PBMCs stored for 48 hours (pathways with changes in > 5 genes)

	Pathways	Genes affected	Functions involved
Up-regulated after storage	Cytokine-cytokine receptor interaction	CXCL4, PDGFRA, TNF, SF21, IL1 β , CCL20, CCL2, CCL4, CCL3, CCL7, CCL23	Cytokine/chemokine stimulation, Apoptosis
	MAPK signaling pathway	PDGFR, G12, NF1, PTP, MKP, c-Myc, TNF, IL1, CD14, Cdc42/Rac	Apoptosis/anti-Apoptosis Stress response
	Regulation of actin cytoskeleton	F2R/CD14, PDGFRA, ACTN, RTK, ITG, Cdc42, G α 12, 13, CFN	Stress response, Cell adhesion
	T cell receptor signaling pathway	CD3 ϵ , CD3 δ , ITK, Rho/Cdc42, PCK1, NFAT, IkB, TNF α	Immune response, Apoptosis
	NK cell mediated cytotoxicity	ICAM1/2, NKG2A/B, 3BP2, SHC, NFAT, TNF α	Apoptosis, Stress response
	Toll-like receptor signaling pathway	CD14, IkB α , TNF α , IL1 β , MIP-1 α , MIP-1 β	Immune response, Cytokine/chemokine Stimulation, Apoptosis
	Insulin signaling pathway	SOCS, PI3K, ERK1/2, EIF4E, AMPK, PKA	Anti-apoptosis
Down-regulated after storage	Fc epsilon RI signaling pathway	BTK, SLP76, PI3K, MKK4/7, ERK, cPLA2	Cytokine/chemokine Stimulation
	Glycan structures-biosynthesis I	MAN2A1, CHST2, GLNAC4S-6ST, B4GALT7, CHST11, GCNT1	Cell structure
	MAPK signaling pathway	NGF, ERK, cPLA2, TAB1, ASK1, MKK4	Anti-apoptosis/Apoptosis Stress response