

Glycosyltransferase and sulfotransferase gene expression profiles in human monocytes, dendritic cells and macrophages

François Trottein · Lana Schaffer · Stoyan Ivanov · Christophe Paget · Catherine Vendeville · Aurélie Cazet · Sophie Groux-Degroote · Suzanna Lee · Marie-Ange Krzewinski-Recchi · Christelle Faveeuw · Steven R. Head · Philippe Gosset · Philippe Delannoy

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Abstract Using a focused glycan-gene microarray, we compared the glycosyltransferase (GT) and sulfotransferase gene expression profiles of human monocytes, dendritic cells (DCs) and macrophages (M ϕ s), isolated or differentiated from the same donors. Microarray analysis indicated that monocytes express transcripts for a full set of enzymes

involved in the biosynthesis of multi-multiantennary branched *N*-glycans, potentially elongated by poly-*N*-acetyl-lactosamine chains, and of mucin-type Core 1 and Core 2 sialylated *O*-glycans. Monocytes also express genes involved in the biosynthesis and modification of glycosaminoglycans, but display a limited expression of GTs implicated in glycolipid synthesis. Among genes expressed in monocytes (90 out of 175), one third is significantly modulated in DCs and M ϕ respectively, most of them being increased in both cell types relative to monocytes. These changes might potentially enforce the capacity of differentiated cells to synthesize branched *N*-glycans and mucin-type *O*-glycans and to remodel cell surface proteoglycans. Stimulation of DCs and M ϕ s with lipopolysaccharide caused a general decrease in gene expression, mainly affecting genes found to be positively modulated during the differentiation steps. Interestingly, although a similar set of enzymes are modulated in the same direction in mature DCs and M ϕ s, cell specific genes are also differentially regulated during maturation, a phenomenon that may sustain functional specificities. Validation of this analysis was provided by quantitative real-time PCR and flow cytometry of cell surface glycan antigens. Collectively, this study implies an important modification of the pattern of glycosylation in DCs and M ϕ s undergoing differentiation and maturation with potential biological consequences.

F. Trottein (✉) · S. Ivanov · C. Paget · C. Vendeville · C. Faveeuw
Inserm, U547,
Lille 59019, France
e-mail: francois.trottein@pasteur-lille.fr

F. Trottein · S. Ivanov · C. Paget · C. Vendeville · C. Faveeuw · P. Gosset
Institut Pasteur de Lille,
Lille 59019, France

F. Trottein · S. Ivanov · C. Paget · C. Vendeville · A. Cazet · S. Groux-Degroote · M.-A. Krzewinski-Recchi · C. Faveeuw · P. Gosset · P. Delannoy
Université Lille Nord de France,
59000 Lille, France

L. Schaffer · S. Lee · S. R. Head
The SCRIPPS Research Institute,
La Jolla, CA, USA

A. Cazet · S. Groux-Degroote · M.-A. Krzewinski-Recchi · P. Delannoy (✉)
UMR 8576, CNRS,
Villeneuve d'Ascq 59655, France
e-mail: philippe.delannoy@univ-lille1.fr

P. Gosset
INSERM, U774,
Lille 59019, France

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Abbreviations

APC Antigen presenting cell
DC Dendritic cell

Ag	Antigen
GSL	Glycosphingolipid
GT	Glycosyltransferase
M ϕ	Macrophage
TLR	Toll-like receptor
sLe ^x	Sialyl Le ^x
GAG	Glycosaminoglycan
ST	Sialyltransferase
PSA	Polysialic acid
HS	Heparan sulfate
qPCR	Quantitative real-time PCR
LPS	Lipopolysaccharide
MFI	Mean fluorescence intensity
GM-CSF	Granulocyte M ϕ -colony stimulating factor

Introduction

Glycosylation of proteins and lipids plays a crucial role in numerous biological processes including the regulation of immune and inflammatory responses (for reviews, [1–6]). During physiological conditions, glycans exert diverse functions on the immune system. By serving as ligands for glycan-binding proteins, such as classical adhesion molecules and lectins, they mediate immune cell differentiation, survival, adhesion, and trafficking [7–13]. During stress or infection, glycans also play a pivotal role by triggering or controlling immune cell signalling, migration, expansion and/or effector functions [10, 14–20]. Glycans exposed on the surface of professional antigen (Ag) presenting cells (APCs) are likely to be critical in many aspects of immune responses. They mediate host-pathogen interactions, influence their tropism and emigration and shape their biological functions after cell-to-cell contact. For instance, glycans play a part in the cross-talk between dendritic cells (DCs), the most potent APCs, and conventional T lymphocytes to modulate the strength and the quality of the acquired immune response [21]. Moreover, interactions of APCs with cells of the innate system, including natural killer cells, are supported by glycan/counter-receptor interactions (for review, [22]). More recently, a new concept has emerged showing that the production of glycolipids (glycosphingolipids, GSLs) by CD1d-expressing APCs is critical to activate Natural Killer T cells, a sub-population of innate/memory non-conventional T lymphocytes (for reviews, [23, 24]). So far, although differentiation and activation of APCs, including monocytes, DCs and macrophages (M ϕ s), are accompanied by programmed remodelling of cell surface (glycosylated) molecules with potentially biologically important consequences, comparative analysis of the expression of genes involved in glycan biosynthesis (essentially

glycosyltransferases, GTs) and modification (mainly sulfo-transferases) has only been reported in DCs [25, 26].

The mononuclear phagocyte system is composed by monocytes, DCs and M ϕ s, which contribute to tissue remodelling and homeostasis, inflammation and immune defence. Circulating monocytes, which constitute ~ 5–10% of peripheral blood leukocytes in humans, give rise to tissue-resident M ϕ s as well as to other specialized cells such as osteoclasts and myeloid DCs [27–30]. Dendritic cells are critical in the induction, expansion and regulation of immune responses (for reviews, [31–34]). Immature DCs principally locate at sites of Ag entry, where they are poorly immunogenic but efficient at capturing Ags through receptor-mediated endocytosis, macropinocytosis and phagocytosis. Upon activation by inflammatory factors and/or microbial components, DCs undergo a complex process of maturation allowing their traffic to T-cell areas of lymphoid tissues through modification of adhesion molecule and chemokine receptor expression. Dendritic cell maturation is also associated with high surface expression of Ag presenting and co-stimulatory molecules and with secretion of chemokines and bioactive mediators such as immuno-stimulatory cytokines. These factors are important to the downstream activation of numerous bystander innate immune cells, such as Natural killer (T) cells, and to promote T and B cell stimulation, expansion and polarisation [31–34]. Although M ϕ s are much less potent in inducing primary immune response relative to DCs, they are crucial players in innate/acquired responses, in particular in immediate early defence against bacterial infection. The most prominent functions of M ϕ s are phagocytosis of intruding microorganisms and necrotic/apoptotic cells, bactericidal activity and rapid secretion of pro-inflammatory cytokines (for review, [27]). Like DCs, activation/maturation of M ϕ s is mediated by engagement of various innate sensors, including Toll-like receptors (TLRs) (for reviews, [35, 36]).

In the present study, we aimed to obtain general information relating to the expression of genes encoding GTs and sulfotransferases in human monocytes, DCs and M ϕ s isolated or differentiated from the same donor. We also aimed to compare the expression profile of these genes in immature *versus* mature (immuno-stimulatory phenotype) DCs and M ϕ s. To this end, we took advantage of a focused gene microarray (glycogene-chip v3, Consortium for Functional Glycomics) using the Affymetrix technology. The glyco-gene-chip v3 is a custom designed GeneChip expression array that utilizes 21 probes for each targeted transcript, ten more than are found on the commercially available GeneChip arrays such as the Hu133 Plus 2.0 array from Affymetrix. The additional probes used on this focused array increase the sensitivity for detection of low abundant transcripts (unpublished results, SRH). In the present study, we report that the differentiation and

maturation processes affected the gene expression profiles of GTs and sulfotransferases in DCs and M ϕ s. Potential functional consequences for these changes are discussed.

Materials and methods

Reagents

Human recombinant GM-CSF was purchased from Peprotech (Rocky Hill, NJ) and IL-4 were from R&D systems (Abingdon, UK). LPS (type 055B5) was purchased from Sigma-Aldrich (St Louis, MI). Anti-CD14-microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany) were used for magnetic cell separation. The FITC-conjugated mouse anti-CD1a, anti-CD16, anti-CD209 (DC-SIGN) and anti-HLA-DR mAbs were obtained from Becton Dickinson (San Diego, CA). The APC-conjugated anti-CD11c and anti-CD86 mAbs as well as the PE-conjugated mouse anti-CD80 and anti-CD14 mAbs were purchased by Becton Dickinson, whereas the anti-CD83 mAb was from Coulter (Miami, Flo). The anti-M ϕ mAb (clone RFD7) was furnished by AbD-Serotec (Abingdon, UK). The anti-G_M3 (clone GMR6), anti-G_D3 (clone S2-566), anti-Le^a (clone 7LE), anti-Le^b (clone 2-25 L), anti-Le^y (clone H18A) and anti-Lewis^x (Le^x, clone 73-30) mAbs were from Seikagaku Corporation (Tokyo, Japan). The anti-sialyl Tn (clone HB-STn1), anti-sLe^a (clone 2D3) and anti-Tn Ag (clone M0896) were from Dako (Glostrup, Denmark) and the anti-sialyl Le^x (sLe^x, clone CSLEX1) from Becton Dickinson. The anti-PSA Ab (clone 735) was kindly provided by Prof. R. Gerardy-Schahn (Medizinische Hochschule, Hannover, Germany). The FITC-conjugated *Sambucus nigra* agglutinin (SNA) lectin was from Vector Laboratories (Burlingame, CA).

Preparation and stimulation of human DCs and M ϕ s

After giving informed consent, blood from healthy volunteers was collected (Etablissement Français du Sang, Lille). Peripheral blood mononuclear cells were prepared by density centrifugation on Ficoll-Paque. Blood monocytes were purified by positive selection over a MACS column using anti-CD14-conjugated microbeads. This purified cell population contained at least 95% CD14⁺ cells. An aliquot containing about 3–5 × 10⁶ monocytes was immediately frozen to prepare RNA. Monocytes were then differentiated into DCs [37, 38] or into M ϕ s [39] by standard procedures. Briefly, monocytes were cultivated at 10⁶ cells/ml for 6 days in RPMI 1640 with 10% heat-inactivated FCS (Invitrogen, Paisley, UK) containing 10 ng/ml IL-4 and 25 ng/ml GM-CSF or GM-CSF alone to obtain myeloid DCs [40, 41] or proinflammatory type I M ϕ s [42, 43],

respectively. At day 3, half of the culture medium was renewed by addition of fresh complete medium containing cytokines. At day 5, DCs and M ϕ s (at least 95% pure, as revealed by flow cytometry) were stimulated or not with LPS (100 ng/ml). Cells were collected after 18 h stimulation to prepare RNA or after 24 h for FACS analysis. Cell death was assessed by trypan blue exclusion and measurement of MTT oxydo-reduction (Sigma) in all culture conditions and neither exceeded 10%.

Microarray analysis of gene expression

Analysis of gene expression was conducted using a custom gene microarray (GLYCOv3 chip) produced by Affymetrix for the Consortium for Functional Glycomics (www.functionalglycomics.org), and containing probe sets for over 1,000 human genes including 199 human GTs and sulfotransferases. In this study, we focused our analysis on the expression of the 175 genes involved in the biosynthesis of *N*-glycans, mucin-type *O*-glycans, glycosaminoglycans, and glycolipids. Five to six independent experiments were performed for each condition. Total RNA was extracted using the Qiagen Mini kit according to the manufacturer's suggested protocol (Qiagen, Inc., Valencia, CA). The quality of the samples was checked with an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). For GT and sulfotransferase expression analysis, total RNA (1 μ g) was amplified and biotin-labelled using the Bioarray MessageAmp II-Biotin Enhanced Kit from Ambion and then hybridized to the GLYCOv3 array. Hybridization and scanning of samples was performed using standard Affymetrix protocols for GeneChip expression arrays based on methods originally described in [44] (protocol available at <http://affymetrix.com>). Chips were scanned using the Affymetrix ScanArray 3,000 using default settings and a target intensity of 250 for scaling. The transcriptional profile was evaluated in independent cell preparations, each derived from a different donor and one chip per biological sample was run. Expression calls indicate whether a gene is detected above background using the GC-matched bins as background measurement. For this purpose the Fisher's Combined P Method [45] as implemented in the R program software (R is available as Free Software under the terms of the Free Software Foundation's GNU General Public License). The Fisher's Combined P method threshold was adjusted so that the expression calls were consistent with the present and absent calls as implemented in the Affymetrix Microarray Suite V5. Intensity values were normalized using Robust Multichip Average (RMA) [46, 47]. The Limma package in the R software was used for the Anova analysis. The fold changes and standard errors were estimated by fitting a linear model for each gene and empirical Bayes smoothing was applied

to the standard errors for all the samples at the same time. Statistics were obtained for transcripts with the multiple testing adjusted (Benjamini-Hochberg) *p*-values to a level of 0.05. Filtering was performed so that probe-sets were eliminated from the results with a fold change of <1.4. Heatmaps were generated by dChip (<http://biosun1.harvard.edu/complab/dchip/>). Raw data files for each of the experiments performed are available at the consortium for Functional Glycomics website (www.functionalglycomics.org/fg) under Resource Request no. 268.

Gene expression analysis by qPCR

Changes in gene expression observed by microarray analyses were verified by qPCR for some genes. Briefly, DNA was synthesized from 1 µg of total RNA with random hexamer primers and Superscript reverse transcriptase (Invitrogen, Cergy Pontoise, France) using standard procedures. cDNAs were used as templates for PCR amplification using the SYBR[®] Green PCR Master Mix (Molecular Probes, Leiden, The Netherlands) and the ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA). Primers listed in Table 1 were designed by the Primer Express Program (Applied Biosystems) and used for amplification in triplicate assays. PCR amplification of GAPDH was performed to control for sample loading and to allow normalization between samples. Δ Ct values were obtained by deducting the raw cycle threshold (Ct values) obtained for β -actin mRNA, the internal standard, from the Ct values obtained for investigated genes. Data are expressed as fold mRNA level increase or decrease compared to the mRNA expression level in non-differentiated (immature DCs or immature M ϕ s vs. monocytes) cells or to non-stimulated cells (mature DCs or mature M ϕ s vs. immature DCs or immature M ϕ s, respectively).

Flow cytometry analysis

Cells were collected in PBS containing 2 mM EDTA at 4°C and labelled as previously described [37]. Cells were incubated for 30 min in aliquots of 2×10^5 cells in 50 µl of PBS containing 2% FCS with or without Abs (10 µg/ml) or the lectin SNA (40 µg/ml). After washing, cells were directly analyzed for the conjugated mAbs. In some cases, cells were incubated with Phycoerythrin-conjugated goat IgG anti-mouse IgG or IgM (Southern Biotechnology, Birmingham, Al). After 30 min incubation, labelled cells were washed and analyzed. Flow cytometry data were acquired on viability-gated cells using a FACSCalibur flow cytometer and analyzed with the CellQuest software system (BD Biosciences). Results are expressed as the mean fluorescence intensity (MFI) obtained with specific mAbs

or lectins minus the value obtained with the isotype control (Δ MFI).

Results

Expression profile of GT and sulfotransferase genes in monocytes

We first analyzed the expression pattern of GT and sulfotransferase genes involved in the biosynthesis of *N*-glycans, mucin-type *O*-glycans, glycosaminoglycans (GAGs), and glycolipids in circulating peripheral blood monocytes. We found that, among the 175 analyzed genes (200 probe set), 90 are significantly expressed in human monocytes (five donors). Table 2 shows the average expression signals of these genes, which are clustered according to the specificities of the enzymes that they encode. As shown in Table 2, transcripts for fucosyltransferases (FucT), and to a lesser extent for glucosyltransferases (GlcT), glucuronosyltransferases (GlcAT) and mannosyltransferases (ManT) are expressed at a lower level compared to those of the other subfamilies. Interestingly, several genes involved in the first steps of *N*-glycosylation, in particular in the formation of the oligosaccharidyltransferase complex such as *DAD1* (probes #92/93), *RNP1* (#94), *RNP2* (#95) and *DDOST* (#96), are highly expressed in monocytes. Concomitantly, genes encoding enzymes involved in both *N*-glycan branching (*i.e.* *MGAT5B* (#34), *MGAT2* (#36), *MGAT4B* (#37/38/41) & *MGAT1* (#40)) and poly-*N*-acetyl-lactosamine chain elongation (*i.e.* *B3GNT1* (#29), *B3GNT2* (#30), *B3GNT8* (#35), *IGNT2* (#31), *B4GALT3* (#22) and *B4GALT4* (#17)) are also well expressed, suggesting that monocytes synthesize *N*-glycans carrying di, tri, and tetra-antennary structures, possibly elongated by poly-*N*-acetyl-lactosamine chains. In parallel, monocytes also highly express *B4GALT5* (#24) and *B4GALT1* (#25), two genes encoding type 2 disaccharide (Gal β 1-4GlcNAc) synthesising enzymes, together with fucosyltransferases (*FUT4* (#6) and *FUT7* (#5)) and sialyltransferase (*ST3GAL6* (#58)) using type 2 disaccharide as acceptor substrate. This indicates that monocytes possess a full set of enzymes for the biosynthesis of Le^x and sialyl (s)Le^x in terminal position of oligosaccharide chains. In agreement with previous reports [48, 49], FACS analysis confirmed expression of sLe^x, but not Le^x, on monocytes (Table 3). At the opposite, the β 1,3-galactosyltransferases (β 3-Gal T1, β 3-Gal T2, β 3-Gal T5 and β 3-Gal T7) involved in the biosynthesis of type I disaccharide unit (Gal β 1-3GlcNAc) are not significantly expressed in monocytes. This correlates with the absence of type I derived blood group Ags, such as Le^a, sLe^a or Le^b, as revealed by FACS analysis

Table 1 Oligonucleotides for qPCR

Gene (Accession #)	Sense primers (5' → 3')	Antisense primers (5' → 3')	Reference
<i>B-actin</i> (NM_001101)	TCC TCA CCC TGA AGT ACC CCA AGC CAC ACG CAG CTC ATT GT		this study ^a
<i>GALNT3</i> (NM 004482.2)	CAC GGC TGT CGT AAG TCC AGA T GAA GCG ACT CCC AGC CAA AT		this study
<i>GBGT1</i> (NM 021996.3)	GCA GGT GGC CAG GGT ATA TG AGC ACC TTG GAC GGC TTG T		[71]
<i>B4GALT5</i> (NM 004776.2)	TCT CTC GTC CTC GCT GCT GTA C CCG AAG CAC CTG CTC ATA AAC C		[71]
<i>B3GNT7</i> (NM 145236)	TCA AAG GCG ACG ATG ACG T TTT GTT GTC TTT CCT GCG AAT G		this study
<i>MGAT4A</i> (NM 012214.1)	CTG TGG AAG TTT TGC CTT TTA AGA G TGA AAT GGG ATT GAG ACT TGG A		[71]
<i>B3GNT2</i> (AB049584)	GCT GGA CCT CAT CGG GAT AA TGC ATA GGG TGG GTA GAG GC		[71]
<i>B3GNT5</i> (NM 032047)	GTG GTG CCC CTC CCA TTA G GCT CCG GCT GTG TAG TCA GG		[71]
<i>ST8SIA4</i> (NM 005668.1)	GAG CAC CAG GAG ACG CAA CT GAG CCA GCC TTT CGA ATG ATT		this study
<i>ST6GAL1</i> (NM 173216.1)	GGG CTC CAA ACT AAC CAT CTC AAA TCC AGG CTT TCT CAC TCC		[72]
<i>CHST2</i> (NM 004267.1)	TCT CTA CGA GCC AGT GTG GCA GAG AGG TCG CAG CGG TAA AGA		[72]
<i>GALNAC4S6ST</i> (NM 014863.1)	TGA TTA TTC ACT GCG CGC CT GCT TGT CAA AAA CGC TGA GCC		this study
<i>GLCE</i> (NM 015554)	TCA TGG AGC ACA GTT ACC A TTG TCG AGG AAT CCC TTA C		this study
<i>FUT4</i> (NM 002033.2)	GAG CTA CGC TGT CCA CAT CAC CGA C CAG CTG GCC AAG TTC CGT ATG		[72]
<i>CIGALT1</i> (NM 020156.1)	CAT CCC TTT GTG CCA GAA CAC C GCA AGA TCA GAG CAG CAA CCA G		[72]
<i>GCNT1</i> (NM 001490.1)	AGG ACG TTG CTG CGA AGG AGA C CCC AGC AAG CTC CAA GTG TCT G		[72]
<i>UGCG</i> (NM 003358.1)	TGG AGG GAA TGG CCG TCT T TTG CCT TCT TGT TGA GGT GTA AT		this study
<i>ST3GAL6</i> (NM 006100.2)	TTT TGA GGA GGA TAT TTG GCT AC AAC AAA CAC TGC CTT CAT TGT AC		[72]
<i>ST6GALNAC2</i> (NM 006456.1)	TCA CCA AGT CAT CGC CTC C TTG GCA CTC TCT GAG CCG T		[71]
<i>ST3GAL5</i> (NM 003896.1)	ATC GGT GTC ATT GCC GTT GT TTC ATA GCA GCC ATG CAT TGA		[71]
<i>ST3GAL1</i> (NM 003033.1)	AGA CGC TCA GGG AAA GGT T GTT ATC ACG CCA AGC AAG		[72]
<i>CHST7</i> (NM 019886.1)	CAC CCG GAC GTT TTC TAC TTG AAG AGC GAA CGC AGC ATG T		[71]

^a Generated from human genomic databases by PrimerExpress (Applied Biosystems)

(Table 3). Polypeptide GalNAc-transferases, the mucin-type *O*-glycan initiating enzymes, are also well produced in monocytes. Seven different enzymes (*i.e.* ppGalNAc T3 (#7), T10 (#8/12), T7 (#9), T11 (#10), T2 (#11), T1 (#14), T6 (#15/16)) are fairly or highly expressed, suggesting a very good capacity for monocytes to synthesise *O*-glycan chains. In parallel, monocytes also express the Core 1 β -Gal T1 (CIGALT1, #18), the Core 2 β -GlcNAc T1 (GCNT1, #28) and several core 1-specific sialyltrans-

ferases (*i.e.* ST6GalNAc IV (#59), ST6GalNAc II (#61), and ST3Gal I (#65), but other core-specific enzymes such as Core 2 β -GlcNAc T3, Core 2 and 4 β -GlcNAc T4 or Core 3 β -GlcNAc T6 are not detected. Moreover, monocytes do not express a transcript for ST6GalNAc I, the enzyme responsible for the synthesis of sialyl-Tn (NeuAc α 2-6GalNAc α 1-*O*-Ser/Thr). These data, together with the absence of Tn (GalNAc α 1-*O*-Ser/Thr) or sialyl-Tn Ags (FACS analysis, data not shown), suggest that

Table 2 GlycoGen Chip V3 analysis of the expression of GTs and sulfotransferases in human monocytes

Probe #	Common name	Acc. #	Av. signal	SD
<i>Fucosyltransferases</i>				
1	FUT10	NM 032664.3	21.45	5.18
2	FUT8, long transcript	NM 004480.1	30.39	13.05
3	FUT11	NM 173540.1	41.19	7.29
4	FUT6	NM 000150.1	96.27	7.56
5	FUT7	NM 004479.2	97.30	36.28
6	FUT4	NM 002033.2	145.46	25.13
<i>N-acetyl-galactosaminyltransferases</i>				
7	GALNT3 (ppGalNAc T3)	NM 004482.2	12.49	5.25
8	GALNT10 (ppGalNAc T10) short	NM 017540.1	48.71	5.87
9	GALNT7 (ppGalNAc T7)	NM 017423.1	100.17	53.70
10	GALNT11 (ppGalNAc T11)	NM 022087.1	166.81	52.88
11	GALNT2 (ppGalNAc T2)	NM 004481	206.76	53.49
12	GALNT10 (ppGalNAc T10) long	NM 198321.2	219.63	59.39
13	GBGT1 (Forssman synthase)	NM 021996.3	344.63	109.72
14	GALNT1 (ppGalNAc T1)	NM 020474.2	352.50	132.88
15	GALNT6 (ppGalNAc T6)	NM 007210.2	411.38	111.46
16	GALNT6 (ppGalNAc T6)	NM 007210.2	456.92	188.67
<i>Galactosyltransferases</i>				
17	B4GALT4 (β 4-Gal T4)	NM 003778	28.48	6.34
18	C1GALT1 (core1 β 3-Gal T1)	NM 020156.1	28.67	10.41
19	B3GALT6 (β 3-Gal T6)	NM 080605	81.46	32.41
20	B4GALT2 (β 4-Gal T2)	NM 001005417	95.45	8.31
21	B4GALT7 (β 4-Gal T7)	NM 007255.1	121.48	26.53
22	B4GALT3 (β 4-Gal T3)	NM 003779.2	198.86	52.57
23	B3GALT4 (β 3-Gal T4)	NM 003782.3	201.39	91.42
24	B4GALT5 (β 4-Gal T5)	NM 004776.2	526.75	230.39
25	B4GALT1 (β 4-Gal T1)	NM 001497.2	802.76	330.15
<i>N-acetyl-glucosaminyltransferases</i>				
26	B3GNT7 (β 3-GlcNAc T7)	NM 145236	28.24	5.50
27	MGAT4A (β 4-GlcNAc T)	NM 012214.1	34.89	11.81
28	GCNT1 (Core 2 β 6-GlcNAc T1)	NM 001490.1	40.69	10.50
29	B3GNT1 (β 3-GlcNAc T1)	NM 006876.1	71.37	10.71
30	B3GNT2 (β 3-GlcNAc T2)	AB049584	111.91	56.62
31	GCNT2 (β 6-GlcNAc T)	NM 145649	131.51	31.69
32	B3GNT7 (β 3-GlcNAc T7)	NM 145236	158.47	17.24
33	DPAGT1 (DoI-P GlcNAc-1-P Tf)	NM 203316.1	163.53	26.14
34	MGAT5B (β 6-GlcNAc T)	NM 198955	176.27	12.51
35	B3GNT8 (β 3-GlcNAc T8)	NM 198540.2	179.20	37.90
36	MGAT2 (β 2-GlcNAc T)	NM 002408.2	223.35	135.92
37	MGAT4B (β 4-GlcNAc T) long	NM 054013	332.19	121.76
38	MGAT4B (β 4-GlcNAc T)	NM 014275.1	342.10	136.02
39	B3GNT5 (β 3-GlcNAc T5)	NM 032047	507.49	379.57
40	MGAT1 (β 2-GlcNAc T)	NM 002406.2	719.10	229.41
41	MGAT4B (β 4-GlcNAc T) long	NM 054013	731.74	149.67
<i>Glucosyltransferases</i>				
42	UGCGL2 (Glc T2)	NM 020121.2	26.33	2.09
43	UGCG (GlcCer synthase)	NM 003358.1	52.92	33.45
44	UGCGL1 (Glc T1)	NM 020120	131.51	21.98

Table 2 (continued)

Probe #	Common name	Acc. #	Av. signal	SD
45	ALG6	NM 013339	134.13	52.34
46	ALG8	NM 024079	233.07	59.42
47	ALG5	NM 013338	328.89	79.66
<i>Glucuronosyltransferases</i>				
48	B3GAT3 (GlcAT I)	NM 012200.2	197.61	52.85
49	CSGLCA-T	AB037823	313.38	99.69
<i>Mannosyltransferases</i>				
50	ALG11	AK025456	68.73	28.44
51	ALG9	NM 024740	71.12	7.34
52	ALG12	NM 024105.1	84.98	16.67
53	ALG1	BC004402.1	117.98	30.92
54	ALG2	NM 033087	187.81	57.14
55	ALG3	NM 005787.1	245.59	34.81
<i>Sialyltransferases</i>				
56	ST6GALNAC3 (ST6GalNAc III)	NM 152996	16.92	8.40
57	ST8SIA4 (ST8Sia IV)	NM 005668.1	48.84	25.40
58	ST3GAL6 (ST3Gal VI)	NM 006100.2	65.26	19.22
59	ST6GALNAC4 (ST6GalNAc IV)	NM 014403.1	83.45	10.08
60	ST3GAL3 (ST3Gal III)	NM 174972.1	87.04	8.37
61	ST6GALNAC2 (ST6GalNAc II)	NM 006456.1	145.59	35.02
62	ST6GALNAC6 (ST6GalNAc VI)	NM 013443.3	158.23	23.76
63	ST6GAL1 (ST6Gal I)	NM 173216.1	296.63	91.38
64	ST3GAL5 (ST3Gal V)	NM 003896.1	374.04	83.90
65	ST3GAL1 (ST3Gal I)	NM 003033.1	455.45	198.19
<i>Sulfotransferases</i>				
66	HS3ST4	NM 006040.1	19.96	2.17
67	GAL3ST4	NM 024637.1	40.32	4.23
68	CHST5	NM 012126.1	51.41	2.55
69	HS2ST1, variant 1	NM 012262	54.39	21.96
70	HS2ST1, variant 2	NM 012262.2	61.76	24.51
71	HS3ST3B1	NM 006041.1	62.90	4.16
72	CHST12	NM 018641.1	115.02	24.86
73	CHST14	NM 130468	158.78	64.82
74	CHST7	NM 019886.1	190.92	91.39
75	CHST13	NM 152889	202.92	44.64
76	NDST1	NM 001543.3	215.88	110.42
77	CHST2	NM 004267.1	245.46	42.84
78	NDST2	NM 003635.1	347.32	80.96
79	CHST11	NM 018413.1	423.72	93.24
80	GalNAc4S6ST	NM 014863.1	1025.46	331.16
<i>GAG copolymerases</i>				
81	EXTL2	NM 001439.1	29.09	6.91
82	CHGN (CS GalNAcT1)	NM 018371	30.29	8.48
83	GALNACT-2 (CS GalNAcT2)	NM 018590	42.25	24.60
84	HAS3 isoform b	NM 138612	49.30	6.65
85	CHGN (CS GalNAcT1)	NM 018371	77.57	41.78
86	EXT2 (HS copolymerase)	NM 000401.2	155.41	41.13
87	EXT1 (HS copolymerase)	NM 000127.1	282.69	105.48
88	CSS1	NM 014918	340.15	172.09

Table 2 (continued)

Probe #	Common name	Acc. #	Av. signal	SD
<i>Others</i>				
89	GLCE (C5-glucuronyl epimerase)	NM 015554	23.88	10.19
90	XYLT2 (Xylosyltransferase II)	NM 022167.1	146.25	53.39
91	C1GALT1C1 (COSMC)	NM 152692	192.27	109.24
92	DAD 1	NM 001344.1	869.50	221.68
93	DAD 1	NM 001344.1	969.50	178.38
94	RNP1 (Ribophorin I)	NM 002950.1	1063.92	283.60
95	RNP2 (Ribophorin II)	NM 002951.2	1145.00	218.46
96	DDOST	NM 005216.3	1522.57	314.08

Relative expression of GT and sulfotransferase genes is expressed as the RMA normalized expression values of five different donors. Genes listed are detected above background by the Fisher's Combined P-value method in at least four donors

CS Chondroitin Sulfate; GAG glycosaminoglycan; HS Heparan Sulfate; SD Standard derivation

monocyte *O*-glycan chains are mainly Core 1 and Core 2 sialylated glycans. This is in line with recent data reporting mass spectrometry *O*-glycan profiling of monocytes [26]. Besides *N*-glycan and mucin-type *O*-linked glycan genes, monocytes express genes encoding for enzymes involved in GAG biosynthesis, including those implicated in the tetrasaccharide linker, chondroitin sulphate and heparan sulfate copolymerases, and for modification enzymes such as 5-epimerase and numerous

sulfotransferases (Table 2). Finally, although the expression levels of the two key GTs controlling the first step of GSL synthesis (glucosylceramide synthase UGCG, #43) and lactosylceramide synthase) are low or undetectable, monocytes transcribe several genes encoding enzymes involved in GSL glycosylation. For instance, in the Globo series of GSLs, the G_{b4} synthase (GBGT1, #13), but not the G_{b3} synthase, is highly expressed in monocytes. In the Ganglio series, the G_{M3} synthase (ST3Gal V, #64) is highly expressed but both

Table 3 Expression of cell surface markers and glycan Ags in monocytes, DCs and Mφs

	Ags	Monocytes		DCs		Mφs	
		Medium		Medium	LPS	Medium	LPS
Cell Markers							
	CD14	45.4±4.8		1.4±0.24	2.2±0.58	3.2±0.58	2.4±0.51
	CD1a	1.2±0.5		451.6±99.7	218.4±53.8	1.8±0.37	2±0.55
	CD209	1.25±0.6		313±42.7	172.3±35.3	2.25±0.63	2.75±0.44
	CD16	0.5±1.1		1±0.2	1±0.3	30.5±9.3	29±10.01
	RFD7	2.1±0.1		0.6±0.4	0.05±0.1	31.3±26.9	9.7±2.5
	CD80	5.7±2.2		31.3±5.7	183.5±30.3	11.33±2.7	76±12.4
	CD86	2.3±0.9		8.71±1.57	276.1±35.05	17.3±3.65	87.9±21.25
	HLA-DR	3.7±1.1		80±8.1	232.7±21.9	66.5±9.6	74.7±13.5
	CD83	0.9±0.4		2.1±0.85	22.5±4.7	0.75±0.48	1±0.71
Glycan Ag							
	Le ^x	5±4.8		1±0.8	0.5±0.4	1.3±0.6	2±1
	sLe ^x	53±5		1509±643	565±181	621±303	224±128
	Le ^a	3.5±1.4		2.2±1.4	1.8±1.6	22.5±17.5	24.3±28.9
	sLe ^a	1.2±0.49		0.3±0.2	0.4±0.2	1.7±1.5	1.1±0.9
	Le ^b	1.25±0.63		1.7±1.5	2.7±2.5	8.5±5.5	5.7±3.1
	Le ^y	2.4±1.4		5.7±4.7	5.4±2.9	21.8±19.3	23.5±19.6
	SNA	577±195		1514±447	1756±585	1531±714	1422±524
	G _{M3}	4.2±3		13.2±4.2	19±11	11±6.2	23±3.6
	G _{D3}	0.17±0.15		1.4±0.8	0.8±1.1	2.1±2.2	3±5.2

Unstimulated (medium) and LPS-activated DCs and Mφs were analyzed by FACS. Results expressed in ΔMFI are the mean ± SEM of four–six experiments

G_{D3} synthase (ST8Sia I), G_{T3} synthase (ST8Sia V) and G_{M2} synthase (β 4-GalNAc T1) are not detected. Together, this indicates that ganglioside biosynthesis is rather limited to the a-series of gangliosides in monocytes.

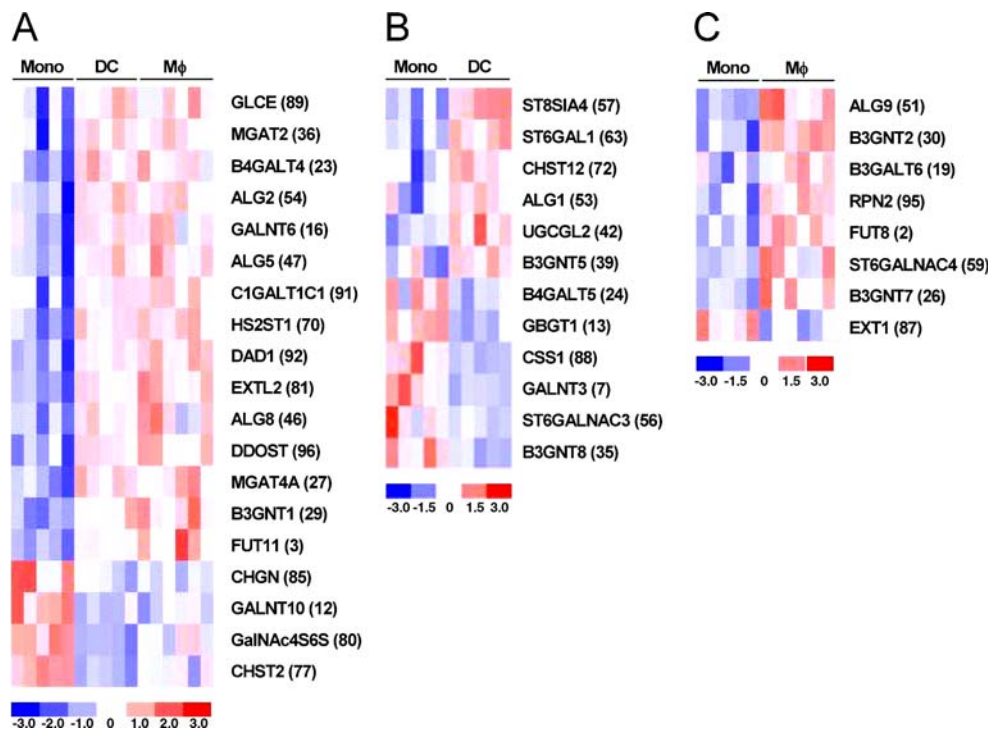
Comparison of GT and sulfotransferase gene expression in DCs and M ϕ s relative to monocytes

In humans, monocytes have the capacity to differentiate into CD11c⁺ MHC class II⁺ DCs in the presence of granulocyte M ϕ -colony stimulating factor (GM-CSF) and interleukin-4 or into M ϕ s in the presence to GM-CSF alone [38, 50, 51]. We investigated whether *in vitro* differentiation of DCs or M ϕ s from monocytes leads to changes in the level of GT and sulfotransferase transcript expression. The quality of the differentiated cells was assessed after selection and differentiation by flow cytometry. As shown in Table 3, clear phenotypic differences between monocytes, DCs and M ϕ s was observed. Unlike M ϕ s and monocytes, immature DCs express the canonical markers CD1a and DC-SIGN (CD209). At the opposite, M ϕ s, but not DCs, produce CD16 and RFD7. Monocytes are strongly positive for CD14, a marker lost during their differentiation, and some cells are also positive for CD16. Furthermore, transcriptomic analysis confirmed that cell-specific markers are expressed by DCs, such as the lectins DEC-205, DC-SIGN (CD209) and DC immunoreceptor (DCIR), and by M ϕ s, such as the scavenger receptors collectin 12 and LOX-1 or are common to both cell types

such as the macrophage mannose receptor (CD206) (data not shown).

As shown in heatmap representation (Fig. 1), in both DCs and M ϕ s, a significant number of GT and sulfotransferase genes (31/90 (34 %) and 27/90 (30 %), respectively) are significantly ($P < 0.05$) changed in their expression levels (fold change > 1.4). Indeed, 21 GT and sulfotransferase transcripts are increased and only ten are decreased in DCs, whilst in M ϕ s, 22 GT and sulfotransferase mRNAs are increased and five are decreased. To validate these changes, we performed quantitative real-time PCR (qPCR) using biological samples (three to five) independent of those used in the gene chips analysis. To this end, we focused on genes with the highest expression variation and/or involved in specific pathways. As Table 4 shows, and among the tested genes, the qPCR analysis confirmed data obtained by the micro-array approach. Strikingly, DCs and M ϕ s exhibit similarities in their pattern of GT and sulfotransferase transcript expression, indicating that the majority of these genes are modulated in the same direction during the differentiation processes (Fig. 1). Among them, several genes coding for enzymes involved in the first steps of *N*-glycan biosynthesis are increased (albeit moderately) in differentiated cells, such as those directly associated to the biosynthesis of the lipid-linked precursor of Asn-linked glycans, the dolichol-PP-oligosaccharide (*ALG1* (#53), *ALG2* (#54), *ALG5* (#47), *ALG8* (#46) and *ALG9* (#51)) and to the transfer of the oligosaccharide onto the nascent protein (*DAD1* (#93) and *DDOST* (#96)). Variation of gene

Fig. 1 Heatmaps showing differential regulation of GT and sulfotransferase gene expression during the differentiation of monocytes into DCs and M ϕ s. Monocytes were cultured to differentiate into either DCs (GM-CSF plus IL-4) or M ϕ s (GM-CSF). Profiles for transcripts differentially expressed in both DCs and M ϕ s (a), only in DCs (b) and only in M ϕ s (c). Heatmap colors represent relative \log_2 expression values median scaled across the samples for each transcript, red showing increased and blue showing decreased expression. Common names and probe #'s from Table 2 are indicated beside the heatmaps. Details for determining these significant differentially transcripts are presented in the Methods section



transcripts are also observed for GTs that selectively act in the Golgi processing of *N*-glycans, such as the *N*-acetylglucosaminyltransferase MGAT2 (#36), MGAT4A (#27), and particularly the β 3-GlcNAc T1 (#29), the latter being essential for the synthesis of poly-*N*-acetylglucosamine chains [52]. These enzymes might contribute to an enforced capacity of differentiated cells to synthesise branched poly-*N*-acetylglucosamine *N*-glycans chains. Interestingly, the polysialyltransferase ST8Sia IV (#57), one of the key enzymes of polysialic acid (PSA) biosynthesis, a structure usually found on neural cell adhesion molecule [53], is over-expressed in DCs. However, in contrast with a recent report describing PSA expression on neuropilin-2 [54], we failed to detect PSA expression on the DC surface (not shown). This contrasting result may be explained by differences in the experimental protocols used to generate DCs, which could result in the lack of neuropilin-2 and therefore of PSA. Differentiation is also accompanied to changes in the expression of GTs involved in *O*-linked glycan synthesis. Compared to monocytes, the gene encoding the polypeptide *N*-acetylglucosaminyltransferase 6 (ppGalNAcT6 or GALNT6, #16), is increased in both DCs and M ϕ s. In parallel, ppGalNAcT10 (GALNT10, #12) is decreased in both differentiated cells and ppGalNAcT3 (GALNT3, #7) in DCs. This indicates that, along with an enforced *N*-type glycosylation, the mucin-type *O*-linked glycosylation might also be modified during DC and M ϕ differentiation. *FUT11*, the gene encoding the putative α 1,3/4-fucosyltransferase 11 (#3) is also increased in both DCs and M ϕ s. This might explain the increased expression of sLe^x in immature DCs (~27 fold compared to monocytes), as indicated by FACS analysis (Table 3), although the expression other enzymes implicated in sLe^x synthesis

(FUT7, ST3Gal III, IV or VI) remains unchanged. Major changes are also found for genes encoding enzymes implicated in proteoglycan synthesis, in particular in the sulfatation of GAGs. Indeed, the HS sulfotransferase HS2ST1 (#70) is increased in both DCs and M ϕ s, whilst the chondroitin 4-sulfotransferase CHST12 (#72) is increased only in DCs. Conversely, transcripts for the *N*-acetylgalactosamine-4-sulfate 6-*O*-sulfotransferase (GalNAc4S6ST, #80), the *N*-acetylglucosamine 6-*O*-sulfotransferase 2 (CHST2, #77) and the chondroitin sulfate *N*-acetylgalactosaminyltransferase 1 (CHGN, #82) are decreased in differentiated cells, relative to monocytes. Finally, transcript for exostosin-like 2 (EXTL2, #81), a copolymerase that transfers GlcNAc and GlcA to the common GAG-protein linkage region to initiate HS synthesis [55], is increased in both DCs and M ϕ s, whilst the heparan sulfate copolymerase EXT1 (#87) is decreased in M ϕ s. In parallel, within the proteoglycan family, a group of genes including agrin, bamacan, glypican 4, decorin and syndecan 2, 3 and 4 are upregulated in both DCs and M ϕ s, as revealed by our micro-array analysis (not shown). Altogether, in agreement with Wegrowski *et al.* [56], DCs and M ϕ s probably exhibit an important remodelling of proteoglycans on their surface.

Comparison of gene expression between DCs and M ϕ s also indicates that a limited number of GT and sulfotransferase genes are cell specific (12 in DCs and 8 in M ϕ s, Fig. 1a and c). In agreement with a recent study reporting a high α 2,6-linked sialic acid density in immature DCs [57], ST6Gal I (#63) is over-expressed in DCs. The enhanced (~3-fold) exposure of α 2,6-linked sialic acid on differentiated cells, relative to monocytes, was confirmed by flow cytometry using the SNA lectin (Table 3). The lactotriaosylceramide (Lc3) synthase (B3GNT5, #24) and

Table 4 mRNA expression of GT and sulfotransferase genes during the differentiation of DCs and M ϕ s, as analyzed by qPCR

# in Table 2	Common name	Gene	Accession number	DC/Monocyte	M ϕ /Monocyte
7	ppGalNAc T3	<i>GALNT3</i>	NM 004482.2	0.18±0.28	0.55±0.25
13	Forssman synthase	<i>GBGT1</i>	NM 021996.3	0.06±0.01	0.53±0.30
24	β 4-Gal T5	<i>B4GALT5</i>	NM 004776	0.44±0.16	0.91±0.32
26	β 3-GlcNAc T7	<i>B3GNT7</i>	NM 145236	1.74±0.75	8.58±6.40
27	β 4-GlcNAc T4a	<i>MGAT4A</i>	NM 012214	7.02±4.79	4.08±0.78
30	β 3-GlcNAc T2	<i>B3GNT2</i>	AB 049584	1.32±0.89	4.54±3.86
39	β 3-GlcNAc T5	<i>B3GNT5</i>	NM 032047	4.29±2.01	0.70±0.46
57	ST8SIA IV	<i>ST8SLA4</i>	NM 005668.1	4.81±1.72	1.42±0.4
63	ST6Gal I	<i>ST6GAL1</i>	NM 173216	2.50±1.56	1.10±0.46
77	Chondroitin-6-keratan-Sulfo T2	<i>CHST2</i>	NM 004267	0.10±0.08	0.52±0.27
80	Chondroitin GalNAc-4- <i>O</i> -sulfate 6- <i>O</i> -sulfo T	<i>GALNAC4S6ST</i>	NM 014863	0.25±0.24	0.76±0.46
89	C5-glucuronyl epimerase	<i>GLCE</i>	NM 015554	2.57±0.96	2.52±1.07

RNAs from resting monocytes or from unstimulated DCs or M ϕ s were harvested after differentiation and mRNA copy numbers were measured by qPCR. Data are normalized to expression of β -actin and are expressed as fold increase over average gene expression in monocytes. Data represent the mean \pm SEM of three to five independent donors

the Forssman synthase (GBGT1, #13), the enzyme that converts the Pk Ag into the Forssman Ag, are significantly increased in DCs, but not in M ϕ s. These latter observations suggest potential differences in the synthesis of globo and (neo)lacto series of GSLs between DCs and M ϕ s, which could have important consequences on the fine tuning of membrane microdomain organization and cell signalling.

Comparison of GT and sulfotransferase gene expression in mature DCs and M ϕ s, relative to immature cells

As a next step, we compared the expression levels of GT and sulfotransferase transcripts in immature *versus* mature DCs or M ϕ s. To this end, cells were stimulated for 18 h with the canonical TLR4 agonist lipopolysaccharide (LPS). As expected, LPS strongly increases the expression of CD80 and CD86 in DCs, and at a lower level in M ϕ s, whilst that of HLA-DR and CD83 was only induced in mature DCs (Table 3). Microarray analysis revealed that the expression of lectins, which are upregulated during the differentiation, is mainly repressed after maturation (not shown), as confirmed by flow cytometry for DC-SIGN (CD209) (Table 3). Thus, LPS treatment results in dramatic changes in DC and M ϕ phenotype.

Compared to immature cells, LPS treatment is accompanied by a variation of some GT and sulfotransferase gene expression, in particular in DCs. Among modulated genes,

23 genes in DCs and 25 genes in M ϕ s are significantly decreased after stimulation, respectively (Fig. 2). On the other hand, in DCs and M ϕ s, respectively, 15 and 6 genes are increased at 18 h compared to resting cells. Most of these variations were confirmed by qPCR on RNA from three to five samples different from those used in the gene chips analysis (Table 5). Strikingly, LPS treatment preferentially down-modulated genes found to be positively regulated during the differentiation steps (nine for DCs and 11 for M ϕ s). As an example, MGAT4A (#27), DDOST (#96), DAD1 (#92), GLCE (#89), HS2ST1 (#69) and B3GNT1 (#29), which are increased during DC and M ϕ differentiation, are decreased in LPS-stimulated DCs and M ϕ s. A similar observation is made for ST6Gal I (#63), which is increased during the differentiation steps, but strongly decreased after LPS treatment. This was however not accompanied by a decreased binding of SNA on mature cells (Table 3). With the notable exception of core 1 β 3Gal T1 (C1GALT1, #18) and ST3GAL1 (#65), which are increased in stimulated DCs, most of the *O*-linked glycan enzymes are decreased after stimulation. In particular, the core 2 β 6 GlcNAc T1 (GCNT1, #28) is decreased in both mature DCs and M ϕ s. Altogether, these observations are in agreement with [25, 26] and indicate that mature cells have a reduced capacity for synthesising mucin-type *O*-glycans, which might be reduced to sialylated core 1. In agreement with the general picture that maturation inversely modulates the expression of genes affected during the differentiation

Fig. 2 Heatmaps showing differential regulation of GT and sulfotransferase gene expression during the LPS-induced maturation of DCs or M ϕ s. Resting DCs or M ϕ s were stimulated with LPS for 18 h. Profiles for transcripts differentially expressed in both DCs and M ϕ s (a), only in DCs (b) and only in M ϕ s (c). For details see legend for Fig. 1. Genes modulated in opposite ways during differentiation and maturation are marked by an asterisk

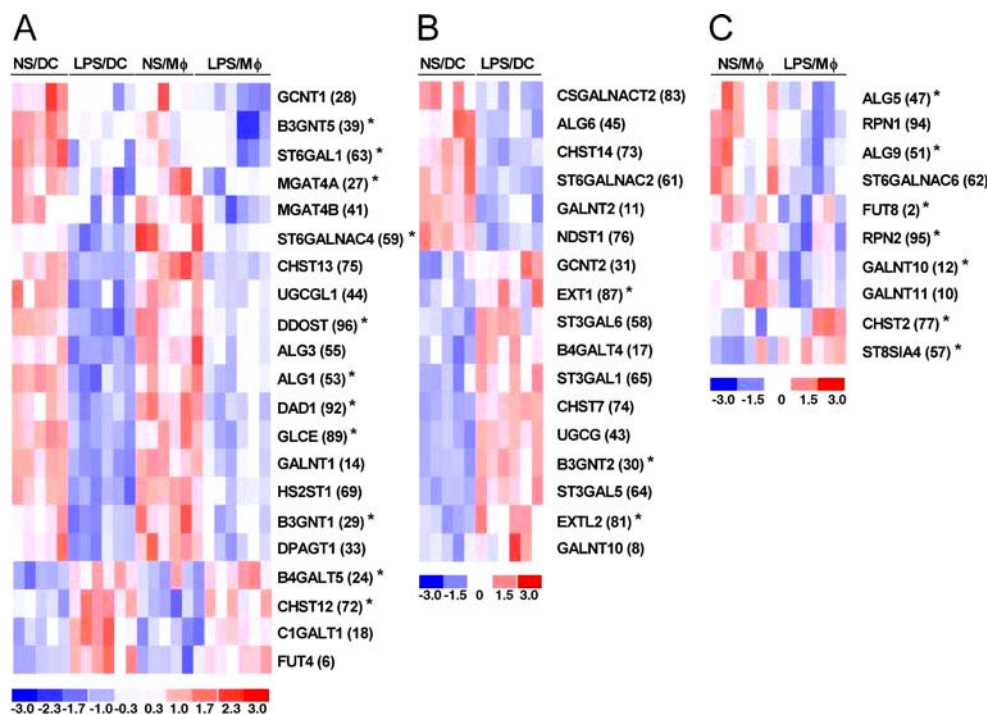


Table 5 mRNA expression of GT and sulfotransferase genes during the maturation of DCs and M ϕ s, as analyzed by qPCR

# in Table 2	Common name	Gene	Accession number	DC LPS/DC NS	M ϕ LPS/ M ϕ NS
6	FUT4	<i>FUT4</i>	NM 002033	3.70 \pm 1.32	4.89 \pm 1.73
18	core1 β 3-Gal T1	<i>C1GALT1</i>	NM 020156.1	0.87 \pm 0.12	3.93 \pm 1.23
24	β 4-Gal T5	<i>B4GALT5</i>	NM 004776	3.62 \pm 1.97	5.90 \pm 1.89
27	β 4-GlcNAc T4a	<i>MGAT4A</i>	NM 012214	0.38 \pm 0.23	0.84 \pm 0.26
28	Core 2 β 6-GlcNAc T1	<i>GCNT1</i>	NM 001490.1	0.44 \pm 0.05	0.69 \pm 0.27
30	β 3-GlcNAc T2	<i>B3GNT2</i>	AB049584	4.05 \pm 1.27	0.96 \pm 0.56
39	β 3-GlcNAc T5	<i>B3GNT5</i>	NM 032047	0.28 \pm 0.13	0.40 \pm 0.38
43	Glucosylceramide synthase	<i>UGCG</i>	NM 003358	2.50 \pm 0.82	2.39 \pm 0.70
57	ST8Sia IV	<i>ST8SIA4</i>	NM 005668.1	2.15 \pm 1.86	3.81 \pm 1.34
58	ST3Gal VI	<i>ST3GAL6</i>	NM 006100.2	3.80 \pm 1.65	1.78 \pm 1.11
61	ST6GalNAc2	<i>ST6GALNAC2</i>	NM 006456.1	0.48 \pm 0.41	2.14 \pm 1.11
63	ST6Gal I	<i>ST6GAL1</i>	NM 173216	0.22 \pm 0.16	0.59 \pm 0.44
64	ST3Gal V	<i>ST3GAL5</i>	NM 003896.1	2.33 \pm 0.75	1.42 \pm 0.50
65	ST3Gal I	<i>ST3GAL1</i>	NM 003033.1	2 \pm 0.42	2.91 \pm 0.83
74	N-acetylglucosamine-6-O-Sulfo T7	<i>CHST7</i>	NM 019886	7.73 \pm 3.62	1.72 \pm 0.14
77	Chondroitin-6-keratan-Sulfo T2	<i>CHST2</i>	NM 004267	1.73 \pm 0.99	2.42 \pm 1.63
89	C5-glucuronyl epimerase	<i>GLCE</i>	NM 015554	0.18 \pm 0.06	0.43 \pm 0.08

RNAs from unstimulated or LPS-stimulated DCs or M ϕ s were harvested after 18 h stimulation and mRNA copy numbers were measured by qPCR. Data are normalized to expression of β -actin and are expressed as fold increase over average gene expression in unstimulated DCs or M ϕ s. Data represent the mean \pm SEM of three to five independent donors

step, genes such as B4GALT5 (#24), EXT1 (#87), CHST12 (#72) and GALNT10 (#8), found to be down-regulated during the differentiation steps, are generally increased in mature DCs or M ϕ s (Fig. 2). However, notable exceptions are observed, for example for genes only modulated during the maturation, but not during the differentiation process. These genes include FUT4 (#6), an α 3-fucosyltransferase involved in the synthesis of selectin ligands, and the core 1 β 3-Gal T1 (#18), for which expression is unchanged during differentiation, but increased in both mature DCs and M ϕ s. We also noticed that some genes including B3GNT2 (#30), the sulfotransferase CHST12 (#72), B4GALT4 (#17), the copolymerase EXTL2 (#81) and ST8SIA4 (#57), found to be enhanced during the differentiation, are also increased during the maturation of DCs and/or M ϕ s (Fig. 2). Finally, LPS treatment leads to cell-specific differences. The first difference concerns the expression of genes, including the pp-GalNAc T2 (#11), the β 3-GlcNAc T2 (#30), the N-acetylglucosamine 6-O-sulfotransferase 7 (CHST7, #74) and 14 (CHST14, #73), the sialyltransferases ST6GalNAc II (#61) and ST3Gal VI (#58) and the chondroitin GalNAc-transferase 2 (CSGALNACT2, #83), which are strongly modified (positively or negatively) in LPS-treated DCs (Fig. 2b), but unchanged in stimulated M ϕ s (Fig. 2c). Similarly, compared to M ϕ s, transcripts for some GSL-specific GTs are enhanced in mature DCs, but not M ϕ s.

These include the glucosylceramide synthase UGCG (#43) and the G_{M3} synthase (ST3GAL5, #64), the later being involved in the synthesis of ganglio series (a, b and c) of GSLs. Flow cytometry analysis, however, revealed no significant increase of G_{M3} cell surface expression after LPS stimulation and no G_{D3} synthesis, in correlation with the low expression of ST8SIA1. Although not fully GSL specific, ST3Gal I (#65), which is implicated in the synthesis of complex gangliosides (G_{M1b}/G_{D1a}/G_{T1b}/G_{Q1c}) is also increased in DCs, but not in M ϕ s.

Discussion

Using a highly sensitive array gathering probes for 175 genes involved in the biosynthesis of N-glycans, O-glycans, GAG and glycolipids, we first analyzed GT and sulfotransferase gene expression profile of human monocytes. Microarray analysis indicated that, among the 175 genes examined, 90 are significantly expressed in human monocytes. Monocytes express transcripts for a full set of enzymes involved in the biosynthesis of multiantennary N-glycans, possibly elongated by poly-N-acetyl-lactosamine chains, and of Core 1 and Core 2 O-linked sialylated glycan chains. Monocytes also express genes potentially involved in sLe^x, but not (s)Le^a, Le^b and Le^y, biosynthesis,

a finding confirmed by FACS analysis. The synthesis of sLe^x on monocytes might be important in the extravasation and infiltration of these cells into peripheral sites, where DCs and M ϕ s differentiate [27]. Monocytes also synthesize genes encoding GTs involved in GAG (heparin and chondroitin sulfate) biosynthesis and encoding sulfotransferases involved in their modification. On the other hand, judging by the low expression levels of genes encoding GT specifically implicated in GSL synthesis, monocytes seem to display a limited capacity to produce glycolipids.

Because differentiation of DCs and M ϕ s is accompanied by programmed remodelling of cell surface glycans with biologically important consequences, we next compared GT and sulfotransferase gene expression in *in vitro* differentiated cells, relative to monocytes. Previous reports have shown that monocyte-to-M ϕ , and possibly monocyte-to-DC differentiation, is associated with modulation of ~1 to 2 % of the global transcriptome [58]. We found that, among genes found to be significantly expressed in monocytes, ~30 % are modulated (mostly increased) in both DCs and M ϕ s. These gene array data were validated by qPCR using biological samples independent of those used in the gene chips analysis. This suggests that GT and sulfotransferase genes are relatively sensitive to the differentiation steps and that DCs and M ϕ s present an enhanced capacity to produce glycans compared to monocytes. Among biosynthetic pathways, it seems that globally, transcripts for enzymes involved in *N*-glycosylation pathway, as well as in mucin-type *O*-glycan and GAG synthesis, are mainly affected. In particular, GT genes involved in *N*-glycan synthesis are generally increased in differentiated cells. The general picture is that transcripts encoding enzymes involved in both initiation and elongation of *N*- and *O*-glycan chains are enhanced during differentiation. Genes encoding endoplasmic reticulum enzymes involved in dolichol cycle or encoding oligosaccharidyltransferase subunits are increased in differentiated cells. In the same time, enzymes involved in both branching and elongation of poly-*N*-acetylglucosamine chains of *N*-glycans, are also augmented. MALDI-TOF analysis of *N*-glycans in DCs has previously shown the presence of sialylated tri- and tetra-antennary *N*-glycans, potentially elongated with poly-*N*-acetylglucosamine and decorated with Lewis-type epitopes [25]. Similarly, several genes encoding polypeptide *N*-acetylgalactosaminyltransferases are increased in differentiated cells suggesting an enhanced synthesis of *O*-glycans. Micro-array analysis also shows the preferential expression of several enzymes involved in type II (Gal β 1-4GlcNAc) terminal unit biosynthesis in monocytes derived-cells. Altogether, these data indicate an enforced capacity of differentiated cells to synthesize type II blood group epitopes exposed at the surface that fits well with the highest sLe^x expression in immature DCs and

M ϕ s, as indicated by FACS analysis. It is possible that, through selectin member interactions, sLe^x might be involved in the migration of DCs and M ϕ s and/or in their interactions with immune cell types (including T cells) [59]. Interestingly enough, specific enzymes involved in the synthesis of other glycan determinants potentially exposed on the cell surface to interact with counter-receptors are positively modulated in differentiated cells. For instance, over-expression of ST6GAL1 transcript in immature DCs, an enzyme that elaborates the terminal sequence NeuAc α 2-6Gal β 1-4GlcNAc on *N*-linked (such as CD45) and *O*-linked glycoproteins, is in agreement with the high α 2-6-linked sialic acid density in immature DCs [57] and could be important in many situations. Indeed, this sialylated sequence represents the glycan ligand for CD22 (Siglec-2), a molecule known to negatively regulate signalling events [15] and implicated in the regulation of Siglec-mediated cell death [60]. Within the proteoglycan family, important changes in the expression of genes encoding enzymes implicated in the sulfatation of GAGs and in the synthesis of proteoglycans (*i.e.* members of the syndecan family) are observed in both DCs and M ϕ s. This might result in important remodelling of proteoglycans on their surface, potentially giving new counter-receptors, relative to monocytes. Collectively, although monocytes, DCs and M ϕ s all belong to the mononuclear phagocyte system, they display a distinct profile of GT and sulfotransferase expression as a result of cell differentiation. Micro-array analysis has revealed clear analogies between DCs and M ϕ s in terms of GT and sulfotransferase transcript expression, without a real clear gene expression signature. Whether the few differences in gene expression between DCs and M ϕ s could account for functional differences is an open question that deserves further investigations.

Since glycosylation-related genes are modulated during stress conditions [61–63], we next investigated whether GT and sulfotransferase transcripts could be modulated in DCs and M ϕ s in response to LPS. Maturation/activation of DCs and M ϕ s results in a profound remodelling of cell surface markers (chemokine receptors, co-stimulatory molecules) with important functional consequences. However, the impact of maturation/activation on glycan synthesis in these cells has only been recently investigated for DCs [25, 26]. To address this question more in depth, cells were stimulated with LPS, one of the most potent TLR activator. Compared to immature cells, LPS treatment was accompanied by a general decrease of GT and sulfotransferase gene expression in both DCs and M ϕ s and mainly affects genes that were found to be modulated during DC and M ϕ differentiation. For instance, in DCs, ~40 % of genes increased during differentiation are decreased during maturation. Notably, excepted GTs involved in the synthesis of sialylated core 1 *O*-glycans, most enzymes involved

in O-linked glycosylation, including the core 2 β 6 GlcNAc T1 (GCNT1), are decreased after LPS stimulation. These findings are in line with recent reports [25, 26], although our data do not confirm the total loss of sLe^x, known to require core 2 structures, on mature DCs. This discrepancy may be explained by experimental differences to induce DC maturation (TNF- α plus PGE₂ vs. LPS). Of note, several genes are also increased during DC maturation such as those involved in GAG biosynthesis (sulfotransferases and co-polymerases). This suggests an enhanced synthesis of HS in mature cells that can be implicated in signaling functions of heparin-binding growth factors and chemokines [55, 64]. Transcripts for enzymes implicated in the production of globo-series (B3GALNT1, GBGT1) and ganglio-series of GSLs (ST3GAL5, ST3GAL1) are specifically enhanced in mature DCs, relative to M ϕ s. This, and the enhanced expression of ceramide glucosyltransferase (UGCG) in DCs, strongly suggests that a profound change of GSL metabolism operates in DCs undergoing maturation. The relevance of this finding is still unknown and deserves further investigations. Glycolipids have been clearly demonstrated to be involved in differentiation, proliferation and migration of neural cells in mammalian central nervous system, and changes in GSL expression in mature DCs might be important in their immunostimulatory functions. Interestingly, TLR-stimulated DCs have recently been shown to generate new or increasing amounts of glycolipid Ags able to activate different subsets of lipid-reactive innate/memory T cells, via the CD1 molecules [65–69]. This pathway is believed to play an important role in innate responses to pathogens and to polarize acquired responses [23, 70]. Altogether, these data suggest that by affecting certain enzymes of the GSL pathway, TLR triggering in DCs could play an important part in innate/acquired responses.

In conclusion, we herein report the GT and sulfotransferase gene expression profiling in DCs and M ϕ s, both in basal and stress (pro-inflammatory) conditions. We show that differentiation (monocyte-to-DC and monocyte-to-M ϕ) and maturation processes are associated with significant changes in GT and sulfotransferase gene expression. Interestingly, although DCs and M ϕ s express a comparable pattern of enzymes, cell specific genes are also differentially modulated during the differentiation and maturation processes, a phenomenon that may sustain functional specificities.

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