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Comprehensive analysis of the transcriptional response of human decidual cells to lipopolysaccharide stimulation

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Abstract

Decidual cells are central to innate immunity at the maternal/fetal interface. We sought to characterize the response of decidual cells to stimulation and then removal of lipopolysaccharide (LPS) using a whole genome approach. Decidual cells were isolated from term unlabored cesarean sections. Cells were stimulated with LPS and RNA isolated both prestimulation and 2 and 24 h post-stimulation. Media were changed and RNA extracted 48 h later. Gene expression was measured using Agilent 44K whole genome microarrays. Data were visualized and interpreted using Ingenuity Pathway Analysis (IPA) software and selected ($n = 5$) target gene expression was verified with quantitative real-time PCR. Genes related to immune function were up-regulated at 2 and 24 h after LPS exposure and then generally returned to baseline or were at least substantially reduced after LPS removal. Pathway analysis also revealed that genes involved in lipid metabolism (specifically cholesterol and steroid biosynthesis), iron metabolism, and the plasminogen system were coordinately altered following exposure to LPS. Our novel, preliminary findings provide insight into possible mechanisms via which the host inflammatory response could contribute to preterm birth and warrant further investigation in preterm samples.

Keywords

Decidual cells; Whole genome analysis; LPS

1. Introduction

Preterm birth contributes significantly to neonatal morbidity and mortality. While spontaneous preterm birth (sPTB) is a heterogeneous disorder, intra-amniotic infection is implicated in a large proportion of early preterm births (Hillier et al., 1988; Watts et al.,

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jri.2011.11.004.

1992). The majority of intrauterine infections are hypothesized to arise from ascending infection (Goldenberg et al., 2000). Infection at the maternal/fetal interface leads to a rapid migration of traditional immune cells such as leukocytes and macrophages. Maternal decidual cells also play a key role in innate immunity at the maternal/fetal interface. Our group and others have demonstrated that decidual cells express toll-like receptors (TLR) 1, 2, 4, and 6, and can elaborate a broad range of cytokines, chemokines and other inflammatory products such as prostaglandins (Dudley et al., 1997; Makhoul and Simhan, 2006; Simhan et al., 2004; Canavan and Simhan, 2007; Lappas et al., 2002).

To date, studies that have characterized decidual cell gene expression in response to inflammatory stimuli have focused on a limited number of inflammatory mediators. Inflammatory stimuli such as lipopolysaccharide (LPS), however, have broad biological consequences. Studies that investigate a limited number of inflammatory mediators are unable to explore the range of biological pathways altered by inflammation. Furthermore, decidual inflammation is hypothesized to occur early in the process of ascending infection. Thus, we hypothesized that a global approach to the characterization of LPS-responsive decidual cell gene expression would improve our understanding of the downstream consequences of maternal/fetal inflammation and identify biological pathways that may become the subject of further functional analyses in this context. Such efforts will ultimately help define possible areas for intervention by improving our understanding of the biology of inflammation at the maternal/fetal interface.

In this pilot study, we sought to characterize the temporal response of decidual cells to stimulation with LPS at the level of the transcriptome. Additionally, we aimed to characterize the gene expression response to the subsequent removal of LPS. In an effort to characterize gene expression in a global manner we used a whole genome microarray approach coupled with a software-based approach for the visualization and functional analysis of the resulting data.

2. Materials and methods

2.1. Tissue collection and cell culture

Decidual cells were isolated from four placentas obtained at the time of cesarean section. All women enrolled had uncomplicated pregnancies with no evidence of chorioamnionitis prior to delivery. Given that labor is an inflammatory stimulus, all samples were obtained prior to clinical signs of labor. Furthermore, for this preliminary study, all samples were obtained at term, as the heterogeneous etiologies of preterm deliveries would have introduced significant confounding factors. Collection of cells was performed in an anonymous fashion without any links to personal identifiers. Our Institutional Review Board granted exemption status for this collection. Decidua was carefully scraped off the chorion using a sterile glass slide. Cells were digested using collagenase Type III and DNase for 2 h at 37 °C. The digestate was filtered, spun at 2200 rpm for 10 min and re-suspended in PBS. A 2-mL suspension was placed over a discontinuous Percoll gradient. Cells in the 20–30% and 30–40% gradient were collected and re-suspended in DMEM. Cells were cultured in DMEM supplemented with 10% FBS at 37 °C with 5% CO₂. Immunohistochemistry was performed on these cells for the prolactin receptor and insulin-like growth factor binding protein-1.

Cells were positive on 10 high-power fields. This is consistent with low contamination from other cell types (Richards et al., 1995; Sutherland and Richards, 1995).

2.2. Treatment with LPS and RNA purification

Cells were grown until 80% confluent and then split for a total of three passages. This yielded four 10-cm plates per placenta. At the start of all experiments each plate contained approximately 1×10^6 cells. Time response experiments were performed with exposure of cells to 10 ng/mL of LPS at baseline, 2 and 24 h (Makhlouf and Simhan, 2006; Simhan et al., 2008; Linjawi et al., 2004). RNA was extracted at each time point. In the last plate the medium was changed to a non-LPS-containing medium at 24 h and RNA was isolated from these cells 48 h later or 72 h after the start of the experiment. Cell viability was confirmed at each time point with trypan blue exclusion. RNA was purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) as per manufacturer's protocol. For all RNA specimens the 260/280 ratio was >1.8 . RNA structural integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

2.3. Agilent oligonucleotide microarrays

Data sets were prepared according to the guidelines of minimum information about a microarray experiment (MIAME) and were deposited in the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>).

For gene expression measurements we used Agilent $4 \times 44K$ whole human genome microarray kits (G4112F; Agilent Technologies) according to the manufacturer's protocol. Microarray analysis was performed on decidual cells obtained from a single placenta following time course exposure to LPS. Follow-up experiments, in which the levels of single transcripts were measured by quantitative real-time PCR (see below), were performed using RNA obtained from the decidual cells of four placental samples. Briefly, 500 ng of total RNA was amplified using an Agilent Low Input Linear Amplification and Labeling Kit, and resultant cRNA was labeled with cyanine-3 (cy-3, 10 mM; PerkinElmer Life and Analytical Sciences, Boston, MA, USA). Cy-3 labeled probes were purified using Qiagen's RNeasy Mini kit (Qiagen) as per the manufacturer's protocol. Sufficient yield and dye incorporation were confirmed using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Arrays were hybridized in duplicate for 17 h at 60 °C under continuous rotation at ~20 rpm. The gasket slide cover slips were removed and the slides washed for 1 min in Agilent Wash Buffer 1 (6× sodium chloride/sodium phosphate/EDTA (SSPE) + 0.005% N-laurylsarcosine). The slides were then washed in Agilent Wash Buffer 2 (0.06× SSPE + 0.005% N-laurylsarcosine) for 1 min followed by 1 min in acetonitrile and then 30 s in Agilent Stabilization and Drying solution. Arrays underwent imaging using the Agilent DNA microarray scanner. DNA microarray feature intensities were measured using Agilent Feature Extraction software version 9.5.2.

2.4. Data analysis

The Agilent microarray data were normalized using the cyclic LOWESS method. We then identified the genes whose expression levels have changed during the time course. That is, a gene is selected if it is differentially expressed between at least one pair of time points. We

used the moderated F tests based on the empirical Bayesian method described in Smyth (2004), to identify the genes differentially expressed over the time course. With a false discovery rate controlled at 5%, we found 10,098 probes targeting 7801 distinct genes. All analyses were performed using the statistical computing package R.

The list of statistically significant differential gene expressions at each time point (2 h, 24 h, 72 h) in relation to baseline (0 h) was used as input into Ingenuity Pathways Analysis (IPA) (*Ingenuity Pathways Analysis*. Ingenuity Pathways Analysis 2009 [cited 2009; Available from: <http://www.ingenuity.com/>]). Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. These genes, called focus genes, were overlaid onto a global molecular network developed from information contained in the Ingenuity Pathways Knowledge Base. Networks of these focus genes were then algorithmically generated by IPA based on their functional connectivity as reported in literature citations.

To increase the stringency of our analysis and provide a gene list suitable for computational biological pathway analysis we further restricted our approach to include only genes within this list that displayed a log ratio change of 0.5 or less. This yielded a total of 1435 genes (2 h), 1465 genes (24 h), and 1513 genes (72 h) that both mapped to genes in the Ingenuity Knowledge Database and also displayed either an increase or decrease in expression over the time course of LPS exposure. These data are presented in Tables S1A–S1C, respectively.

The functional and canonical pathways analysis of a network identified the biological functions and canonical pathways that were most significant to the genes in the network. The network genes associated with biological functions and/or diseases in the Ingenuity Pathways Knowledge Base were considered for the analysis. For analyses of canonical pathways, a ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway is displayed. For both functional and canonical pathways, Fisher's exact test was used to calculate a *p* value. The list of significant *p* values was corrected for multiple testing by the Benjamini–Hochberg (B–H) method (Benjamini and Hochberg, 1995).

2.5. Quantitative real-time PCR

Each RNA sample was converted to cDNA using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA) as per the manufacturer's protocol. TaqMan gene expression assays for the following genes: IL1B (Hs00174097_m1), IL6 (Hs00174131_m1), TNF (Hs00174128_m1), SLC40A1 (Hs00205888_m1), and STAR (Hs00264912_m1) as well as for the endogenous control GUSB (Hs00939627_m1) were purchased from Applied Biosystems. For each real-time PCR reaction, 1 µL of cDNA, 1 µL of gene expression assay, and 10 µL of TaqMan gene expression master mix were combined with water in a well on the reaction plate for a total volume of 20 µL. Thermal cycling conditions for all gene expression assays were identical with an initial 2-min incubation at 50 °C to activate the uracil N-glycosylase, followed by a 10-min denaturation step at 95 °C, and then 40 cycles for 15 s at 95 °C and 1 min at 60 °C. Each reaction was analyzed in triplicate, run against the endogenous control, and calibrated against time point *t* = 0. This eliminated any differences in input DNA variation and allowed the data to be read as a relative quantity in

which all sample values were recorded as fold-changes relative to time point $t = 0$ (Livak and Schmittgen, 2001). The quantitative real-time PCR QPCR reactions were amplified and the data analyzed using the 7900HT Sequence Detection System (Applied Biosystems).

3. Results

3.1. Whole genome expression after LPS stimulation

Expression levels of 33291 gene transcripts interrogated by 43376 probes were determined by oligonucleotide microarray analysis. We identified a total of 7801 differentially expressed genes across the entire time course. Table 1 contains the 50 most differentially expressed genes identified across the entire data set including all time points. Table S1 contains the entire list of statistically significant differentially expressed transcripts. Most gene expression is increased, rather than reduced, following exposure to LPS. As expected, chemokines and cytokines dominate the list of the most up-regulated genes including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL10, CXCL11, CXCL12, CXCR4, CXCR7, IL1A, IL1B, IL6, IL8, IL10, IL11, and IL12. Other genes induced by exposure to LPS are involved in the inhibition of apoptosis such as BIRC3, DDIT4, and TNFAIP3. The majority of these genes are acutely upregulated by 2 h (Table S2A) following exposure to LPS, and in many cases this increase in expression is sustained at 24 h (Table S2C).

It is harder to identify coordinated alterations in the expressions of genes that are down-regulated following 2 h of LPS exposure, although, as shown in Table S2B, mRNA levels of the APC2, BMF, DDIT4, and RHOH genes are reduced after a 2-h exposure to LPS, suggesting a possible antiapoptotic and antiproliferative phenotype. Not surprisingly, an analysis of genes down-regulated at 24 h compared with 2 h (Table S2D) suggests a return toward baseline levels of many of the genes encoding inflammatory and immune modulators that were acutely up-regulated within between 2 h and baseline. This trend is continued after the removal of LPS, as shown in Table S2E. Of interest are the most down-regulated genes identified after removal of LPS, which are outlined in Table S2F. These include a number of growth factors (FIGF, HGF, and IGF1) and extracellular matrix-associated genes (MMP11 and COL4A3) as well as genes that play a role in cell migration (GNAT1, FIGF, PLA2G3, and HGF).

3.2. Biological pathway analysis after LPS stimulation

We then used IPA software to identify biological pathways and networks that were over-represented among LPS-responsive genes. Exposure to LPS coordinately modulated genes involved in immune function and inflammation and significantly altered the cytokine canonical pathway. Fig. 1 illustrates how the expressions of genes in this pathway are altered over the time course. Similarly, “cellular movement” was identified as the most significantly altered functional gene network (Fig. 2). This network was populated almost entirely by immunomodulatory genes. Following the removal of LPS for 48 h, the expression levels of genes related to immune response and cellular movement largely returned to or near baseline levels.

When performing the functional analyses using IPA software we were particularly interested in biological pathways that were altered with both stimulation and removal of LPS. In addition to the broad increase in proinflammatory cytokine transcription at 2 h, we observed an increase in the expression levels of genes involved in steroid metabolism and cholesterol synthesis at 48 h after removal of LPS (Fig. 3). These include steroidogenic acute regulatory protein (STAR) and other key genes including SREBF2, which is a master regulator of lipid homeostasis (Eberle et al., 2004) and DHCR7, which is important in cholesterol biosynthesis (Moebius et al., 1998).

3.3. Quantitative real-time PCR

Quantitative real-time PCR (QPCR) was used to confirm gene expression changes. We focused the PCR analysis on five genes: IL1B, IL6, TNF, SLC40A1, and STAR. These genes were chosen because they represented both anticipated and potentially novel findings. The QPCR data are depicted in Fig. 4. The microarray data suggested that the three cytokine genes, IL1B, IL6, and TNF, were most up-regulated at 2 h after LPS stimulation and decreased progressively thereafter and this was confirmed by the QPCR data. The QPCR data also confirmed the LPS-responsive expression patterns of SLC40A1 and STAR by decidual cells in response to stimulation by LPS.

4. Discussion

Ascending infection is an important cause of intraamniotic infection. The decidual cell plays an important role in the innate immune response to ascending infection at the maternal/fetal interface. We sought to characterize gene expression by decidual cells following both stimulation with LPS and subsequent removal of LPS. The use of microarray technology and computational pathway analysis highlights the range of genes and pathways involved in the inflammatory response at the maternal/fetal interface and allows exploration of possible novel findings. These are discussed in greater detail below.

First, this work confirms findings from prior studies demonstrating that decidual cells are capable of responding to LPS with elaboration of a broad range of chemokines and cytokines. Importantly, the time course of cytokine and chemokine gene expression identified by our microarray analysis is well supported by the QPCR data.

Second, we identified solute carrier family 40 (SLC40A1) as a significantly down-regulated gene 2 h after stimulation with LPS. SLC40A1 encodes ferroportin, an iron export protein that plays a key role in regulating tissue and plasma levels of iron (Pietrangelo, 2004). Access to iron is crucial for bacterial growth and genes involved in iron metabolism may modulate inflammation at the maternal/fetal interface. Interestingly, other investigators have identified genes involved in iron metabolism as potentially important modulators of the inflammatory response in the genital tract. For example, Xu et al. (2008) demonstrated up-regulation of LCN2, a member of the lipocalin family that has bacteriostatic effects through reduced access to iron, in the pregnant rat cervix exposed to medoxy-progesterone-acetate in an inflammation-induced model of preterm labor. Iron is also hypothesized to play a role in oxidative stress. A recent review by Sakata et al. (2008) has highlighted the role of iron-dependent oxidative stress in the pathogenesis of preterm birth. Given these data, the

regulation of iron metabolism, both with regard to modulation of bacterial growth as well as pathways involved in oxidative stress, warrants further research.

Furthermore, we found evidence for LPS-responsive alterations in the plasminogen system with both SERPINE1 and SERPINB2 showing increased expression following LPS treatment. This is potentially significant given the association of the plasminogen system with the biological changes that accompany parturition (Norwitz et al., 2007). Also of interest was the LPS-responsive increase in the expression of the endothelin 1 gene (EDN1) observed after 2 h exposure. This is significant given the recent observation that the endothelin-converting enzyme-1/endothelin-1 pathway plays a critical role in inflammation-associated premature delivery in a mouse model (Wang et al., 2008).

Finally, lipid metabolism emerged as an important pathway both after stimulation of decidual cells with LPS and after removal of LPS from decidual cell cultures. In keeping with prior observations, the genes related to lipid metabolism that were up-regulated immediately after stimulation of LPS were pro-inflammatory cytokines involved in the production of prostaglandins (Saji et al., 2000; Shoji et al., 2007; Roman et al., 2006). Interestingly, after LPS stimulation we also observed the increased expression of genes involved in steroid hormone production (Fig. 3), including STAR, which controls the rate-limiting step in the production of steroid hormones by transporting cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane (Miller, 2008; Wang et al., 1998; Kallen et al., 1998). To our knowledge this is a novel finding in decidual cells, potentially linking inflammation to the regulation and production of steroid hormones. Traditionally, endometrium or the pregnant equivalent decidua, is thought to respond to steroid hormones, but not produce steroid hormones. Non-pregnant endometrium, however, has been shown to produce low concentrations of STAR (Tsai et al., 2001). Furthermore, endometriotic implants produce roughly 10 times the amounts of steady-state STAR compared with eutopic endometrium and are capable of producing steroid hormones *de novo* (Tsai et al., 2001). Production of estradiol by decidua could be particularly relevant. *In vitro* estrogen can stimulate prostaglandin F2 and an excess of estrogen induces gap junctions and oxytocin receptors in myometrium (Garfield et al., 1980; Alexandrova and Soloff, 1980; Schatz and Gurpide, 1983). Sun et al. (2003) have further demonstrated that PGE2, a prostaglandin that can be stimulated by cytokines, mediates increased transcription of STAR. Given our findings, this work from the endometriosis literature may be relevant to decidua and provides an interesting link between inflammation and steroid production not previously described in pregnancy. The relevance of locally produced steroid hormones on gestational tissues warrants further research.

Our study is limited by sample size. Importantly, we ran replicate arrays that yielded consistent data. Furthermore, our primary aim was to explore novel biological pathways involved in the inflammatory response of decidual cells over time. We were not interested in inter-individual variation in inflammatory response where a large sample size would be crucial. However, we were able to clearly replicate the quantitative PCR data for all genes in multiple samples (Fig. 4). These data strongly support the accuracy and reliability of the primary microarray data by confirming that the observed gene expression changes represent a general organized cellular response to LPS and are not sample-specific artifacts. A second

potential limitation of our study is the focus on one single cultured cell type. However, as a first step in characterizing the transcriptomic response to biologically relevant inflammatory stimuli, it is vital to employ a reductionist approach. This is a hypothesis-generating study and as such our goal is to build upon our observations by performing increasingly more comprehensive and systematic analyses in this, and related, model system(s).

Additionally, our study examined a single inflammatory stimulus: LPS. Toll-like receptor 4 (TLR4) recognizes LPS motifs found on many Gram-negative bacteria as well as *Mycoplasma hominis* – all organisms implicated in preterm birth. The different cells implicated in uterine immunity, however, are capable of responding to a number of different pathogen-associated molecular patterns through various toll-like receptors (TLRs) and thus our system models the specific response of bacteria that signal through TLR4. Emerging data suggest that the different TLRs lead to varied inflammatory responses. Thus, as part of our future studies we hope to expand our investigation to include other inflammatory stimuli (Thaxton et al., 2010).

Finally, given the methodological importance of performing our preliminary study in non-pathological decidual cells, all our samples are derived from term women. The inflammatory response of term decidua may differ from preterm decidua, limiting the relevance of these pathways to preterm labor. In future studies we plan to expand our work to preterm birth samples with well-characterized etiologies.

In summary, using microarray technology combined with systems-level computational tools to analyze gene expression of the human decidual cell in response to LPS stimulation we were able to identify novel pathways that may prove important in infection- and inflammation-mediated preterm birth. The nature of whole genome investigations is hypothesis-generating only; our preliminary findings will require further work for confirmation in preterm birth samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Ingenuity pathways analysis network showing relative enrichment of cytokine/chemokine/chemotaxis-related gene expression up- and down-regulation at the 2-h time point. For every gene transcript depicted in the network diagram (node), its corresponding relative expression at 2 h, 24 h, and 72 h (-fold change relative to baseline) is illustrated in the inset heat map. Green denotes down-regulation, while red denotes up-regulation. Solid lines between nodes indicate direct molecular interaction reported in the Ingenuity Pathways Knowledge Base between connected transcripts and/or gene products; dotted lines between nodes indicate an

indirect functional interaction between transcripts and/or gene products as reported in the Ingenuity Pathways Knowledge Base.

Fig. 2. Canonical pathway diagram for role of cytokines in mediating communication between immune cells from the IPA Knowledge base is overlaid with gene expression-fold change values at (A) 2 h, (B) 24 h, and (C) 72 h. Green denotes down-regulation, while red denotes up-regulation. Grayed-out icons indicate that gene transcript was expressed, but did not meet the 0.5 log-fold cut-off criterion.

Sterol/Cholesterol Synthesis and Regulation

Fig. 3.

Ingenuity Pathways Analysis network showing relative enrichment of sterol/cholesterol-related gene expression up- and down-regulation at the 2-h time point. Numbers associated with each gene transcript (node) denote -fold change at the 2-h time point. For every gene transcript depicted in the network diagram, its corresponding relative expression at 2 h, 24 h, and 72 h (-fold change relative to baseline) is illustrated in the inset heat map. Green denotes down-regulation, while red denotes up-regulation. Solid lines between nodes indicate direct molecular interaction reported in the Ingenuity Pathways Knowledge Base between connected transcripts and/or gene products; dotted lines between nodes indicate an indirect functional interaction between transcripts and/or gene products as reported in the Ingenuity Pathways Knowledge Base.

Fig. 4.

Quantitative PCR of decidual cells stimulated with lipopolysaccharide (LPS). Four different decidual cell samples were stimulated with LPS for 0, 2, and 24 h. LPS was removed and samples were incubated for an additional 48 h ($t = 72$ h). Gene expression of six different genes (IL1B, IL6, TNF, SLC40A1, and STAR) was determined by QPCR at each time point for each sample, and all sample values for each target gene were recorded as-fold changes relative to time point $t = 0$. Each panel A–D represents QPCR data from one decidual cell sample, tested for expression levels of multiple genes, as indicated. Each gene contains four data points $t = 0$ (no bar since all values are relative to this), $t = 2$ h (green), $t = 24$ h (orange), and $t = 72$ h (blue). Error bars represent standard deviation between replicates.

Table 1

The top 50 most differentially expressed genes across the entire data set.

Gene name	Description	h0	h2 2 h of LPS exposure	h24 24 h of LPS exposure	h72 48 h after LPS removal	adj.P.Val
CXCL3	Chemokine (C-X-C motif) ligand 3	6.27	8343.15	551.71	7.38	1.17E-05
CXCL1	Chemokine (C-X-C motif) ligand 1	291.73	194269.90	56981.28	570.18	2.11E-05
IL1B	Interleukin 1, beta	40.43	2831.73	2069.57	36.66	5.54E-05
CXCL2	Chemokine (C-X-C motif) ligand 2	120.23	55582.62	3317.81	74.66	9.23E-05
BIRC3	Baculoviral IAP repeat-containing 3	94.94	5003.50	1437.95	75.45	9.27E-05
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer inhibitor, alpha	17218.13	150310.35	62473.54	22424.36	0.00011
MMP11	Matrix metalloproteinase 11 (stromelysin 3)	26299.88	18575.97	16630.12	116225.35	0.00012
CCL3	Chemokine (C-C motif) ligand 3	335.55	4659.32	1598.16	110.21	0.00012
CSF2	Colony stimulating factor 2	7.20	2598.14	729.56	6.74	0.00013
DDIT4	DNA-damage-inducible transcript 4	9009.78	2550.41	10856.58	18489.05	0.00014
HSD11B1	Hydroxysteroid (11-beta) dehydrogenase 1	862.86	949.74	5878.02	2345.38	0.00014
TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	67.68	4079.71	576.07	70.86	0.00014
COL14A1	Collagen, type XIV, alpha 1 (undulin)	409.04	342.83	342.84	3083.69	0.00014
EGR3	Early growth response 3	180.22	1373.22	91.68	141.80	0.00014
ATP8B4	ATPase, Class I, type 8B, member 4	46.81	50.57	138.65	487.44	0.00015
EPHA7	Ephrin type-A receptor 7 precursor	6.27	7.03	7.21	52.87	0.00015
DUSP2	Dual specificity phosphatase 2	848.44	2985.09	649.37	354.32	0.00015
CCL7	Chemokine (C-C motif) ligand 7	81.92	148.64	372.99	49.12	0.00017
ABCA6	ATP-binding cassette, sub-family A (ABC1), member 6	1510.37	1223.70	1240.93	4652.36	0.00017
ICAM1	Intercellular adhesion molecule 1	1189.54	5848.93	9934.65	2295.44	0.00018
SOD2	Superoxide dismutase 2, mitochondrial	3763.03	8618.06	37272.55	5846.25	0.00018
IL6	Interleukin 6	283.89	7337.61	2677.35	515.72	0.00018
NUAK2	NUAK family, SNF1-like kinase, 2	1432.72	6044.83	1868.06	935.32	0.00018
CHODL	Chondrolectin	4151.35	2998.31	3977.01	26971.51	0.00019
HBEGF	Heparin-binding EGF-like growth factor	1224.13	9248.17	1433.29	485.16	0.00019
BDKRB1	Bradykinin receptor B1	1326.66	4209.02	10465.38	2974.10	0.00020
DACHI	Dachshund homolog 1	331.27	342.02	285.41	1670.64	0.00021

Gene name	Description	h0	h2 2 h of LPS exposure	h24 24 h of LPS exposure	h72 48 h after LPS removal	adj.P.Val
TNF	Tumor necrosis factor (TNF super family, member 2)	55.86	422.79	64.64	57.10	0.00022
SLC40A1	Solute carrier family 40 (iron-regulated transporter), member 1	3644.68	1838.45	1705.33	5541.65	0.00022
NPPB	Natriuretic peptide precursor B	927.83	1730.86	1559.64	187.62	0.00022
FAM101A	Family with sequence similarity 101, member A	144.77	302.94	711.70	86.80	0.00022
SYNPO2L	Synaptopodin 2-like	470.29	623.72	369.77	86.51	0.00022
IL4I1	Interleukin 4 induced 1	342.83	598.12	6414.20	630.63	0.00023
PTX3	Pentraxin-related gene, rapidly induced by IL-1 beta	658.85	11825.24	6250.99	667.63	0.00023
HES1	Hairy and enhancer of split 1	1980.64	4180.86	1939.81	525.49	0.00023
ADRB2	Adrenergic, beta-2-, receptor, surface	218.66	499.40	249.64	50.38	0.00024
SERPINE1	Homo sapiens serpin peptidase inhibitor, clade E	8705.96	20827.94	11548.35	2613.79	0.00015
CXCL12	Homo sapiens chemokine (C-X-C motif) ligand 12	3489.26	4366.23	5234.28	16067.07	0.00017
ICAM1	Homo sapiens intercellular adhesion molecule 1	1264.66	6103.49	10403.66	2370.32	0.00017
EDN1	Homo sapiens endothelin 1	4521.24	13883.91	2265.82	873.73	0.00017
CHODL	Homo sapiens chondrolectin	4151.35	2998.31	3977.01	26971.51	0.00019
MMP9	Homo sapiens matrix metalloproteinase 9	5479.92	8042.03	18705.52	3616.55	0.00021
NPPB	Homo sapiens natriuretic peptide precursor B	927.83	1730.86	1559.64	187.62	0.00022
CCL4	Homo sapiens chemokine (C-C motif) ligand 4	58.77	2250.25	338.62	48.62	0.00024
CLDN1	Homo sapiens claudin 1	310.54	992.74	1542.49	223.74	0.00024
IL32	Homo sapiens interleukin 32	238.60	452.08	2268.32	326.12	0.00024
SERPINB2	Plasminogen activator inhibitor 2 precursor	2484.42	5914.42	8179.63	1566.93	0.00025
MYC	Homo sapiens v-myc myelocytomatosis viral oncogene homolog (avian)	3313.46	6350.42	3531.59	1518.58	0.00026
KYNU	Homo sapiens kynureninase (L-kynurenine hydrolase)	315.65	582.03	1114.11	238.10	0.00026
LONRF2	Homo sapiens LON peptidase N-terminal domain and ring finger 2	685.54	1756.31	418.53	1143.33	0.00026

Numbers in columns h0, h2, h24, and h72 are signal units of expression assigned by the Agilent data extraction software.