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LPS Elicits a Much Larger and Broader Inflammatory Response than *E. coli* Infection within the Hippocampus of Neonatal Rats

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Abstract

An immune challenge during the neonatal period can significantly affect the development of the nervous and immune systems, such that long-term abnormalities in immune function and behavior persist into adulthood. Given that immune activation and individual cytokines have been linked to the etiology of many developmental neuropsychiatric disorders, a complete characterization of the neonatal immune response within the brain is warranted. In this study, rats were treated peripherally on postnatal day (P) 4 with either a live *Escherichia coli* (*E. coli*) infection or lipopolysaccharide (LPS), two common models of neonatal immune activation. Inflammatory gene expression was measured within the hippocampus 2 and 24 hours later. We determined that *E. coli* and LPS produce very distinct inflammatory profiles within the brain. Infection with *E. coli* produced a robust, yet relatively IL-1 pathway focused activation of the neonatal immune system within the brain, while LPS produced a very broad and robust immune response within the brain. This analysis also identified common inflammatory genes up-regulated by both *E. coli* and LPS treatment.

Keywords

neonate; *E.coli* infection; lipopolysaccharide; hippocampus; cytokines; chemokines

Introduction

Perinatal infection and immune activation are proposed to be underlying factors in the etiology of many neurodevelopmental disorders, including autism and schizophrenia [1,4,5,14,25,32,33,35,38,40]. As such, it is important to fully understand the neonatal immune system and how it affects the brain, during health and disease processes. Research focusing on the effects of neonatal immune activation uses a variety of manipulations, from live viral and bacterial infections, to single cytokine and direct LPS stimulation of the immune system. We have reported enduring neuroinflammatory changes within the brains of rats as a consequence of infection with live *E. coli* on P4 [7,13]. These changes are linked to a number of behavioral changes later in life, including social and cognitive impairments [10]. Neonatal LPS challenge in rats also elicits long-term changes in physiology and behavior [24,39]; however, as more research emerges, it has become clear that there are fundamental differences in the biochemical and behavioral consequences of LPS and *E. coli*

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infection in neonatal rodents, suggesting that the initial immune response to the two challenges may be quite distinct. Thus, we sought to provide a preliminary characterization of cytokine, chemokine, and toll-like-receptor (TLR) signaling molecule expression within the neonatal brain following *E. coli* or LPS treatment. We present evidence for similarities as well as striking differences in the neonatal immune response elicited by these common immune challenges, with the hope that this information will provide the groundwork for future mechanistic studies aimed at understanding the long-term consequences of early-life immune activation.

Materials and Methods

Animals

Adult male and female Sprague–Dawley rats (55–65 days) were obtained from Harlan (Indianapolis, IN) and housed in same sex pairs in polypropylene cages with *ad libitum* access to food and water. The colony was maintained at 22 °C on a 12:12-hour light-dark cycle (lights on at 0700 EST). Males and females were paired into breeders. Female breeders were visually examined daily for confirmation of pregnancy, and male breeders were removed from cages prior to the day of birth (P0). Sentinel animals were housed in the colony room and screened periodically for the presence of common rodent diseases; all screens were negative. All experiments were conducted with protocols approved by the Duke University Institutional Animal Care and Use Committee.

Bacterial culture

Escherichia coli culture (ATCC 15746; American Type Culture Collection, (Manassas, VA) vial contents were hydrated and grown overnight in 30 ml of brain–heart infusion (Difco Labs, Detroit, MI) at 37°C. Cultures were aliquoted into 1 ml stock vials supplemented with 10% glycerol and frozen at −20°C. One day before injections, a stock culture was thawed and incubated overnight in 40 ml of BHI at 37°C. The number of bacteria in cultures was read using a microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) and quantified by extrapolating from previously determined growth curves. Cultures were centrifuged for 15 min at 4000 rpm, the supernatants were discarded, and the bacteria were re-suspended in the dose-appropriate volume of sterile Dulbecco's PBS (Invitrogen Corp., Carlsbad, CA).

Neonatal injections

We selected a single time point of exposure in this study to eliminate the potential impact of different developmental stages on the immune response produced within the brain. On P4, male pups were injected subcutaneously (30G needle) with either 0.1×10^6 colony forming units (CFU) of live bacterial *E. coli*/g suspended in 0.1 ml PBS, 0.1 ml PBS alone, or 50 μg/kg lipopolysaccharide (LPS) derived from *Escherichia coli* (serotype 0111:B4; Sigma, St. Louis, MO, USA) in 0.1 ml PBS. The dose of LPS chosen (50 μg/kg) is the lower of two commonly used doses in the literature, and produces well-characterized long-term changes in physiology and behavior in rodent models [43–45]. The dose of *E.coli* was chosen based on previous reports from our lab which show significant long-term effects on immune and behavioral responses into adulthood [7,8,11,13]. Between 1300 and 1500 hours, pups were separated from the mother and placed into a clean cage with bedding, injected individually, and returned to the mother as a group within 5 min. Pups within the same litter received the same treatment due to concerns of possible cross-contamination with *E. coli*. However, each experimental group was populated using only 2 pups from a single litter (n = 9 litters) in order to avoid litter effects. Brains were collected either 2 or 24 hours later and flash frozen in isopentane. These time points were selected based on previous literature indicating that inflammatory gene expression following *E.coli* infection peaks at around 24 hours, while the

inflammatory gene expression following LPS treatment is elevated much earlier, around 2 hours postadministration [7,27,34,47].

Hippocampal quantitative real-time PCR

Brains were sectioned (60 μm) on a Leica cryostat at −20°C, and tissue punches were excised from the dorsal hippocampus (plate 27 – 35 from the neonatal brain atlas [36]) using a brain punch tool (1 mm diameter). We selected hippocampus based on our previous work with neonatal *E. coli*, in which long-term changes in cytokine expression, neurogenesis, and glial activation are observed [3–6,10]. Tissue punches were stored at −80°C until assayed for gene expression. RNA was isolated from hippocampal tissue using the TRIzol method [15], and DNASE-treated. Complimentary DNA (cDNA) was synthesized from 500 ng of isolated RNA using the RT^2 First Strand Kit (Cat No C - 03) SABiosciences / Qiagen (Frederick, MD). Gene expression was measured using quantitative real-time PCR with primers designed to measure rat inflammatory cytokines, chemokines and receptors (SABiosciences/Qiagen; Cat. No PARN-011) or the signaling molecules of the TLR receptor pathway (PARN-018) in combination with the RT^2 SYBR® Green qPCR Master Mix (Cat. No PA-010) from SABiosciences/Qiagen (Frederick, MD) following the manufacturer's protocol.

Data Analysis and Statistical Analysis

A threshold amplification cycle number (C_t) was determined for each gene within the linear phase of the amplification plot. Standard deviations calculated from the Ct values obtained across biological replicates ranged from 0.0 – 1.5 cycles, and the reproducibility within samples has a correlation coefficient of approximately 0.97. The ΔC_t was calculated for each gene using [$\Delta C_t = C_t^{\text{Gene of Interest}} - C_t^{\text{Average of housekeeping genes}}$]. The average ΔC_t value was determined for each gene across three biological replicates. To obtain enough RNA, two pups from the same litter were assigned to an experimental treatment and pooled as a single sample. Therefore, one sample represents 2 pooled rats from the same treatment and the same litter. As mentioned above, no more than two pups per litter were used in a single treatment group, each of which was populated using several different litters. Thus overall, 36 offspring from 9 litters were used for these experiments. Statistical significance between groups was determined using a two-tailed Student t-test of the ΔC_t values from control and experimental samples, using $\alpha<0.05$ as the criterion for significance.

The $\Delta\Delta C_t$ was determined for each gene across two treatment groups, one being the control group (group 1) and one being the experimental group (group 2) [$\Delta\Delta C_t = \Delta C_t$ (avg. group 2) − ΔC_t (avg. group 1)]. Group 1 was represented by PBS treated samples (2 or 24 hours) and Group 2 was represented by the *E. coli* treated group (2 or 24 hours) or the LPS treated group (2 or 24 hours). The fold change was calculated for each gene from group 1 to group 2 as $2^{-\Delta\Delta CT}$. A gene was reported here if the fold-change was greater than +1.9 or less than −1.9; and the t-test determined that $p < 0.05$. These standards are those suggested by SABiosciences that can be replicated using alternate real-time PCR analysis.

Results

Inflammatory Gene Expression within the Neonatal Hippocampus after E. coli Infection

Two hours after *E. coli* treatment, 18 genes were significantly up-regulated within the hippocampus (Table 1A), including genes within the Interleukin (IL) -1 family [Caspase 1, IL-18, IL-1 receptor type 1 and IL-1 receptor type 2] and genes in the IL-6 family [IL-6 receptor α and IL-6 signal transduction protein]. Two chemokines, of the C-C motif, and their receptors were significantly up-regulated 2 hours after infection, including Ccl4 and its receptor Ccr5, as well as Ccl7 and its receptor Ccr1. Five chemokines, of the C-X-C motif,

were up-regulated at this time point, including Cxcl1 and Cxcl2, Cxcl9 and Cxcl10, and Cx3cl1.

Twenty-four hours after *E. coli* treatment, only 8 genes were significantly up-regulated and one gene was significantly down-regulated within the hippocampus (Table 1B). Caspase 1 and the IL-1 receptor type 1 remained up-regulated. IL-1β and IL-1α were also up-regulated at 24 hours. IL-10 was increased nearly 8-fold at this time point, consistent with an anti-inflammatory phase of resolution of the immune response [25]. Ccl3 was significantly down-regulated, while Ccl2 and Ccl12 were both significantly up-regulated at this time. Cluster of differentiation (CD) 14 was also up-regulated more than 2-fold.

Inflammatory Gene Expression within the Neonatal Hippocampus after LPS Treatment

Two hours after LPS treatment, 26 inflammatory genes were significantly up-regulated and 12 genes were significantly down-regulated within the neonatal hippocampus (Table 2A). LPS produced a robust chemokine response, as Ccl7, Cxcl1, Cxcl10, and Cxcl2 were all up-regulated 15-, 18-, 6- and 20- fold respectively.

The expression levels of IL-1β, IL1f6, and IL-1 receptor type 2 were significantly up-regulated, whereas IL-1 receptor type 1 was significantly down-regulated following LPS. IL-6 and its receptor, IL6rα, were also significantly up-regulated at this time point. TNFα was significantly up-regulated while two of its receptors, TNF receptor (super family member) 1a and 1b were significantly down-regulated. Other genes significantly down-regulated by LPS treatment included the Toll interacting protein, Tollip, and Bcl6. Fos was also up-regulated 18-fold at this time.

Twenty-four hours after LPS treatment, 24 inflammatory genes were significantly up-regulated and 2 genes were significantly down-regulated within the neonatal hippocampus (Table 2B). Six of the genes up-regulated by LPS treatment at 2 hours remained up-regulated 24 hours later, including Ccl2, Ccl7, Cxcl11, and Cxcl6, IL1β and the IL6 receptor α. Caspase 1 was up-regulated nearly 11-fold, while IL-1β, IL-1α, and IL18 were all up-regulated approximately 3-fold.

CD80, CD86, CD14 and CD180 were all up-regulated 24 hours after LPS treatment. Mitogen activated protein kinase kinase kinase (Map3k) 7 and Ubiquitin-conjugating enzyme E2N, were also significantly up-regulated.

Discussion

We have determined that the immune response produced within the neonatal brain after a live *E. coli* infection is markedly different from that produced by LPS. *E. coli* produced a robust, yet relatively IL-1 family-focused activation of the immune system within the hippocampus, whereas LPS produced a much broader immune response within the same brain region. The findings presented here are novel as we have simultaneously analyzed the expression level of many cytokines, chemokines, and TLR signaling molecules, obtaining a broad characterization of the immune response generated within the neonatal brain. To our knowledge, this is also the first report comparing the neonatal immune response produced by *E. coli* and LPS.

Several labs have demonstrated that neonatal immune activation produces enduring abnormalities in the brain, exaggerated responses to subsequent immune or stress challenges, and differences in behavior in adulthood [see [10] for review]. Our lab has extensively characterized the long-term effects of a neonatal *E. coli* challenge on the brain and behavior in rodents, which has both similarities to and differences from neonatal LPS

models. Whereas both models cause enduring microglial activation [6,7,13,21], neonatal LPS decreases fever production [20], increases corticosterone production, and increases anxiety in adulthood [31,42]. In contrast, neonatal *E. coli* infection produces sensitized fever responses, decreased corticosterone responses to stress, and has no effect on anxiety in adulthood [9,11,12].

What might account for such differences in the long-term effects produced by these two models of immune activation? Following LPS treatment, twenty-six genes were up-regulated within the neonatal brain including genes within the IL-1 family, the IL-6 family, the Interferons, and the TNF family. Notably, the Interferon and TNF pathways were relatively unaffected following an *E. coli* challenge. In contrast, *E. coli* infection significantly affected many genes within the IL-1 family, with a striking convergence of gene expression on this pathway by 24 hours. These data confirm previous analyses of cytokines and chemokines within the brain [7], and complement data implicating IL-1β as a critical molecule underlying the adult cognitive impairments [6,7]. Caspase 1, which cleaves the precursor forms of IL-1β and IL-18 into their active forms, was significantly up-regulated 2 and 24 hours after an *E. coli* infection and to a greater extent than any other analyzed gene affected by *E. coli*. Caspase 1 was also upregulated to an identical level 24 hours after LPS. Thus, Caspase 1 may be a common mechanism between the two immune challenges.

The observed changes in small inducible chemokines were perhaps the most marked differences between LPS and *E. coli*. As one example, Ccl7 was up-regulated nearly 16-fold 2 hours after LPS treatment but only 4-fold following *E. coli* infection. Ccl7 has two known receptors, Ccr1 and Ccr3. Two hours after *E. coli* infection, Ccr1 was significantly up-regulated, while 2 hours after LPS treatment, Ccr3 was significantly up-regulated. Thus, Ccl7 may be a common molecule underlying the response to distinct immune challenges, whereas the response may be tailored to the specific challenge by differences in its receptor expression.

Another novel finding of this data set includes the difference in expression of TLR signaling molecules following LPS or *E. coli* challenge. LPS treatment increased the expression of CD14, CD180, CD80, and CD86, potentially increasing the sensitivity of TLR4 to endotoxin and thus further enhancing cytokine release [28]. Other signaling molecules affected by LPS included MAP3K7, Fos, and Bcl6, which taken collectively suggest that LPS treatment is affecting TLR4 down-stream signaling to enhance the transcription of other genes critical to the immune response [28]. In contrast, CD14 was the only TLR signaling molecule that was up-regulated following an *E. coli* infection. Interestingly, CD14 expression was increased to a similar level by either an LPS challenge or an *E. coli* infection and as such, may also be a common mechanism by which disparate immune challenges cause similar long-term changes in neuroimmune function.

These gene expression data confirm previous independent analyses of cytokine and chemokine gene expression and protein in the neonate following either *E.coli* or LPS treatment [7,34], yet these data also reveal profound differences in the immune response produced by these two immune challenges. The larger and broader response to LPS was surprising, given that *E. coli* is a replicating pathogen. However, these data are similar to a recent report that the fetal immune response to maternal IL-6 injection is actually more robust than the immune response produced by the viral infection itself, of which IL-6 is a primary mediator [26]. Similarly, LPS is more effective than other forms of bacteria (gram-negative) at inducing sickness behavior and cytokine/chemokine expression within the brain [23,37]. It is possible that counter-regulatory (e.g., anti-inflammatory) mechanisms are

recruited in response to live infections, but are circumvented by purified challenges such as LPS or cytokines.

The differences in the immune response produced by *E. coli* and LPS may also be due to differences in the concentration of LPS molecules available for recognition by peripheral immune cells and/or CNS microglia. If true, then the differences in gene expression between LPS and *E.coli* may involve a critical difference in mechanism downstream of antigen presentation. Moreover, E. coli infection may recruit multiple microglial pattern recognition receptors and pathogen-associated molecular patterns involved in phagocytosis and the inflammatory response beyond just LPS and TLR4 alone (e.g. TLR9, CR3, MHC II, and Fcγ (Fcg) immunoglobulin receptors Fcgr1 and Fcgr3a) [41]. These questions remain to be explored in future studies.

We believe these data illustrate that both LPS and *E. coli* infection models provide an interesting perspective on immune function when taken in the context of early neurodevelopment. Rats at this age have a very permissible blood-brain-barrier (BBB) and, therefore, both LPS and *E.coli* have direct and rapid access to the brain from the periphery. In addition, P4 represents a point of development characterized by a remarkable level of amoeboid microglial cell infiltration from the blood stream, ventricles, and meninges into the tissue [17,18,29,46]. As these cells reach their final destination within the brain and develop into mature microglia, they express a fundamentally distinct morphology and biochemistry from that of fully mature/ramified microglia found in the adult brain [see [16] for review]. The hippocampus, in particular, has a high number of microglia with an activated/amoeboid morphology at P4, and this may significantly influence the inflammatory responses of these cells when compared to responses produced in the adult brain. In addition, microglial cells participate in many developmental processes within the CNS including synapse formation, pruning, and apoptosis [2,3,19,22,30]; and as such, the inflammatory factors evoked by an early-life immune challenge, such as those presented here, can ultimately have profound effects on the neural and immune development occurring at that time.

In conclusion, much remains to be explored about the developing immune system and its interactions with the developing brain. We anticipate these data might broaden the scope of future investigations to include the inflammatory cytokines and chemokines reported here. While these data suggest that care should be taken in generalizing the consequences of purified LPS to infection itself, we have also identified some potentially common immune factors that may guide future research aimed at understanding how the incidence of seemingly disparate infections in humans has been positively linked with the incidence of neuropsychiatric disorders. As such, both models presented here remain powerful tools to examine neonatal immune activation and its effects on brain and behavior in adulthood.

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Table 1

Inflammatory Gene Expression within the Hippocampus of P4 males 2 and 24 hours after *E.coli* infection

P4 male pups were treated with either PBS or *E.coli* infection and brains were collected either 2 or 24 hours after injection (n = 3 samples/treatment group at each time point). RNA was extracted from the hippocampus and quantitative real-time PCR determined that **(A)** at 2 hours, 18 genes were significantly up-regulated when compared to PBS treated controls; and **(B)** at 24 hours, 8 genes were significantly up-regulated and 1 gene was significantly down-regulated when compared to PBS treated controls. Genes highlighted in bold are similarly activated by *E.coli* and LPS (see Table 2). Genes highlighted in italics were affected at 2 (**A**) and 24 hours (**B**).

A. Inflammatory Gene Expression 2 hr after E. coli Treatment

Gene Symbol	Description	Accession No.	Fold-Change from PBS control	p - value
Casp1	Caspase 1	NM_012762	+5.98	0.009263
Ccl4	Chemokine (C-C motif) ligand 4 / MIP-1β	NM_053858	+1.95	0.013928
Ccl7	Chemokine (C-C motif) ligand 7 / MCP-3	NM_001007612	+4.10	0.002167
Ccr1	Chemokine (C-C motif) receptor 1	NM_020542	+3.80	0.001394
Ccr10	Chemokine (C-C motif) receptor 10	XM_343968	+2.29	0.005063
Ccr5	Chemokine (C-C motif) receptor 5	NM_053960	+3.01	0.025221
Cx3cl1	Chemokine (C-X3-C motif) ligand 1 / Fractalkine	NM_134455	+2.10	0.002171
Cxcl1	Chemokine (C-X-C motif) ligand 1 / GRO-KC	NM_030845	+2.41	0.028021
Cxcl10	Chemokine (C-X-C motif) ligand 10	NM_139089	+2.46	0.031596
Cxcl2	Chemokine (C-X-C motif) ligand 2	NM_053647	+3.10	0.016091
Cxcl9	Chemokine (C-X-C motif) ligand 9	NM_145672	+2.58	0.007728
Il13ra1	Interleukin 13 receptor, alpha 1	NM_145789	+2.40	0.000645
Il18	Interleukin 18	NM_019165	+2.81	0.004295
Il1r1	Interleukin 1 receptor, type I	NM_013123	+2.16	0.005082
Il1r2	Interleukin 1 receptor, type II	NM_053953	+1.90	0.044168
Il6ra	Interleukin 6 receptor, alpha	NM_017020	+2.78	0.000335
Il6st	Interleukin 6 signal transducer	NM_001008725	+2.18	0.003293
Tnfrsf1b	Tumor necrosis factor receptor superfamily, member 1b	NM_130426	+2.05	0.012533

B. Inflammatory Gene Expression 24 hr after E. coli Treatment

Gene Symbol	Description	Accession No.	Fold-Change from PBS control	p - value
Casp1	Caspase 1	NM_012762	+10.78	0.044
Ccl12	Chemokine (C-C motif) ligand 12 / MCP-5	XM_213425	+3.33	0.03079
Ccl2	Chemokine (C-C motif) ligand 2 / MCP-1	NM_031530	+5.76	0.00094
Ccl3	Chemokine (C-C motif) ligand 3 / MIP-1α	NM_013025	−2.47	0.0343
Cd14	CD14 molecule	NM_021744	+2.31	0.00429
Il10	Interleukin 10	NM_012854	+8.12	0.00016
Il1a	Interleukin 1 alpha	NM_017019	+3.93	0.02459
Il1b	Interleukin 1 beta	NM_031512	+1.98	0.04738
Il1r1	Interleukin 1 receptor, type I	NM_013123	+2.73	0.01057

Table 2

Inflammatory Gene Expression within the Hippocampus of P4 males 2 and 24 hoursafter LPS treatment

P4 male pups were treated with either PBS or LPS and brains were collected either 2 or 24 hours after injection (n = 3 samples per treatment group at each time point). RNA was extracted from the hippocampus and quantitative real-time PCR determined that **(A)** at 2 hours, 26 genes were significantly up-regulated and 12 genes were significantly down-regulated when compared to PBS-treated controls; and **(B)** at 24 hours, 24 genes were significantly up-regulated and 2 genes were significantly down-regulated when compared to PBS treated controls. Genes highlighted in bold are similarly activated by *E.coli* and LPS (see Table 1). Genes highlighted in italics were affected at 2 (**A**) and 24 hours (**B**).

A. Inflammatory Gene Expression 2 hr after LPS Treatment

Gene Symbol	Description	Accession No.	Fold-Change from PBS control	p - value
Bcl6	B-cell CLL / lymphoma 6	XM_221333	−4.68	0.00035
Ccl11	Chemokine (C-C motif) ligand 11	NM_019205	+2.75	0.05255
Ccl12	Chemokine (C-C motif) ligand 12 / MCP-5	XM_213425	+3.84	0.000902
Ccl17	Chemokine (C-C motif) ligand 17 /	NM_057151	−2.04	0.005814
Ccl2	Chemokine (C-C motif) ligand 2 / MCP-1	NM_031530	+8.29	0.027037
Ccl7	Chemokine (C-C motif) ligand 7 / MCP-3	NM_001007612	+15.85	0.011647
Ccr3	Chemokine (C-C motif) receptor 3	NM_053958	+1.95	0.040844
Ccr4	Chemokine (C-C motif) receptor 4	NM_133532	+1.90	0.015109
Ccr6	Chemokine (C-C motif) receptor 6	NM_001013145	+2.02	0.032638
Csf2	Colony stimulating factor 2 (granulocyte-macrophage)	XM_340799	+4.04	0.019204
Csf3	Colony stimulating factor 3 (granulocyte)	NM_017104	+2.77	0.011047
Cxcl1	Chemokine (C-X-C motif) ligand 1 / GRO-KC	NM_030845	+18.08	0.000483
Cxcl10	Chemokine (C-X-C motif) ligand 10	NM_139089	+6.69	0.017281
Cxcl11	Chemokine (C-X-C motif) ligand 11	NM_182952	+6.53	0.002613
Cxcl12	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	NM_022177	−3.52	0.000271
Cxcl2	Chemokine (C-X-C motif) ligand 2	NM_053647	+20.49	0.003258
Cxcl6	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	NM_022214	−2.87	0.030606
Fos	FBJ osteosarcoma oncogene / c-fos	NM_022197	+17.05	0.009266
Ifna1	Interferon-alpha 1 / IFN alpha 1	NM_001014786	+2.22	0.004677
Ifnb1	Interferon beta 1, fibroblast / IFN b	NM_019127	+3.47	0.001946
Il10	Interleukin 10	NM_012854	+3.26	0.031186
Il10ra	Interleukin 10 receptor, alpha	NM_057193	−2.00	0.038764
Il16	Interleukin 16	XM_218851	−3.34	0.003645
Il1b	Interleukin 1 beta	NM_031512	+3.56	0.000657
Il1f6	Interleukin 1 family, member 6	XM_231099	+2.71	0.051029
Il1r1	Interleukin 1 receptor, type I	NM_013123	−1.90	0.014067
Il1r2	Interleukin 1 receptor, type II	NM_053953	+2.36	0.004793
Il2rg	Interleukin 2 receptor, gamma	NM_080889	−1.95	0.007798
Il3	Interleukin 3	NM_031513	+2.67	0.053324
IL6	Interleukin 6	NM_012589	+2.93	0.003175

A. Inflammatory Gene Expression 2 hr after LPS Treatment

Gene Symbol	Description	Accession No.	Fold-Change from PBS control	p - value
Il6ra	Interleukin 6 receptor, alpha	NM_017020	+2.07	0.002653
Itgb2	Integrin beta 2	XM_001069791	−2.21	0.001508
Lta	Lymphotoxin alpha (TNF superfamily, member 1)	NM_080769	+4.88	0.004546
Nfkbia	Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, alpha	XM_343065	+2.40	0.000263
Tnf	Tumor necrosis factor (TNF superfamily, member 2)	NM_012675	+4.33	0.000179
Tnfrsf1a	Tumor necrosis factor receptor superfamily, member 1a	NM_013091	−1.83	0.033447
Tnfrsf1b	Tumor necrosis factor receptor superfamily, member 1b	NM_130426	−2.79	0.041099
Tollip	Toll interacting protein	XM_341961	−2.99	0.019193

B. Inflammatory Gene Expression 24 hr after LPS Treatment

Gene Symbol	Description	Accession No.	Fold-Change from PBS control	p - value
Casp1	Caspase 1	NM_012762	+11.13	0.000366
Ccl2	Chemokine (C-C motif) ligand 2 / MCP-1	NM_031530	+5.14	0.002545
Ccl5	Chemokine (C-C motif) ligand 5 / RANTES	NM_031116	+3.12	0.013004
Ccl7	Chemokine (C-C motif) ligand 7	NM_001007612	+6.06	0.008139
Ccl9	Chemokine (C-C motif) ligand 9	NM_001012357	+2.14	0.038714
Ccr1	Chemokine (C-C motif) receptor 1	NM_020542	+6.05	0.008251
Ccr5	Chemokine (C-C motif) receptor 5	NM_053960	+8.15	2.86E-05
Cd14	CD14 molecule	NM_021744	+2.24	0.006013
Cd180	CD180 molecule / Ly78	XM_226731	+2.45	0.017965
Cd80	Cd80 molecule	NM_012926	+2.32	0.002781
Cd86	CD86 molecule	NM_020081	+2.98	0.015176
Cx3cl1	Chemokine (C-X3-C motif) ligand 1 / Fractalkine	NM_134455	+2.19	0.006843
Cxcl11	Chemokine (C-X-C motif) ligand 11	NM_182952	+3.86	0.010901
Cxcl6	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	NM_022214	−2.12	0.019716
Cxcl9	Chemokine (C-X-C motif) ligand 9	NM_145672	+2.27	0.014916
Hmgb1	High mobility group box 1	NM_012963	+2.20	2.2E-06
Il16	Interleukin 16	XM_218851	−2.26	0.002948
Il18	Interleukin 18	NM_019165	+3.09	0.002811
Il1a	Interleukin 1 alpha	NM_017019	+3.37	0.029365
Il1b	Interleukin 1 beta	NM_031512	+3.42	0.030272
Il6ra	Interleukin 6 receptor, alpha	NM_017020	+4.84	2.3E-06
Il8ra	Interleukin 8 receptor, alpha Mitogen activated protein	NM_019310	+3.97	0.015444
Map3k7	kinase kinase kinase 7 (Tak1)	XM_232855	+2.14	0.005586
Tlr3	Toll-like receptor 3	NM_198791	+2.56	0.000266
Tlr5	Toll-like receptor 5	XM_223016	+4.02	0.02792
Ube2n	Ubiquitin-conjugating enzyme E2N	NM_053928	+2.94	0.012831