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Neuroinflammation facilitates LIF entry into brain: role of TNF and NFκB

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Abstract

Leukemia inhibitor factor (LIF) is a proinflammatory cytokine mediating a variety of CNS responses to inflammatory stimuli. During lipopolysaccharide (LPS)-induced inflammation, blood concentrations of LIF increase, correlating with lethality of sepsis. Circulating LIF crosses the blood-brain barrier (BBB) by a saturable transport system. Here we determine how this transport system is regulated in neuroinflammation. Using transport assays that quantify the influx rate and volume of distribution of LIF in mice, we show that LPS facilitated the permeation of LIF from blood to brain without compromising the paracellular permeability of the BBB as determined by co-administration of fluorescein. Concurrently, gp130 (shared by the interleukin-6 family of cytokines), but not gp190 (the specific receptor for LIF) or CNTF-Rα (unique receptor for ciliary neurotrophic factor which also uses gp130 and gp190), showed increased levels of mRNA and protein expression in cerebral microvessels from the LPS-treated mice. The upregulation of gp130 by LPS was at least partially mediated by vascular tumor necrosis factor receptor (TNFR)1 and TNFR2. This was shown by elevated TNFR1 and TNFR2 mRNA and protein in cerebral microvessels after LPS and by the absence of the LPS effect on gp130 in knockout mice lacking these receptors. The increase of gp130 was also abolished in mice lacking the p50 unit for nuclear factor (NF)-κB. This indicates a role for NFκB signaling in the upregulation of gp130 and increase of LIF transport across the BBB. The results show that neuroinflammation by LPS induces endothelial signaling and enhances cytokine transport across the BBB.

Keywords

blood-brain barrier; LIF; LPS; TNF; NFκB; neuroinflammation

Introduction

Neuroinflammation is a syndrome present in many types of disorders affecting the CNS. A commonly used animal model for neuroinflammation involves induction by lipopolysaccharide (LPS), a prototypical endotoxin that promotes the induction of proinflammatory cytokines by acting on CD14 and toll-like receptor 4 (TLR4). Leukemia inhibitory factor (LIF) may be involved in the effects of LPS. This is shown by enhanced induction of tumor necrosis factor α (TNF) and interleukin (IL)-6 in response to LPS in LIF knockout mice (35). After intravenous or intracerebroventricular injection, an anti-LIF antibody can reduce LPS-induced plasma adrenocorticotropin (ACTH) release. This suggests that LIF participates in the actions of LPS both in the periphery and CNS to induce neuroendocrine changes (34).

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LIF and TNF also interact with each other in bacterial infection and sepsis (10). Reciprocal induction and inhibition of the cytokines can occur in a state-dependent manner. Mouse models indicate that exogenous LIF protects against lethal endotoxemia involving a major role for TNF (2;32). Elevated blood concentrations of LIF, as seen in patients with meningococcemia or septic shock, correlate with higher mortality (31). The role of the CNS response in such actions is not yet clear.

LIF is a member in the IL6 family of cytokines that share the gp130 signal converter. LIF binds specifically to two type-I hematopoietic cytokine receptors, LIFR (gp190) and gp130. LIFR is the specific receptor for LIF and induces LIF signaling by the Janus kinase (JAK)/Signal transducer and activator for transcription (Stat) pathway. The gp130 receptor is shared by members of the interleukin (IL)-6 family of cytokines. Under basal conditions, gp130 displays a wide distribution in both neurons and glial cells inside the brain (33). Inflammation can further induce gp130 as shown by in-situ hybridization. The pattern of gp130 induction differs from that of the IL6 receptor and shows a different response to LPS and IL1 β . Specifically, gp130 mRNA expression is somewhat more restricted than that of IL6R and is increased in the organum vasculosum laminae terminalis (OVLT, a circumventricular organ) and throughout the endothelia of the brain capillaries of LPS-treated rats, but it is not affected by IL1 β treatment (30). The induced expression of gp130 mRNA in the microvascular endothelium after LPS stimulation suggests that the BBB may respond to circulating cytokines that bind to gp130.

The blood-brain barrier (BBB) allows selective permeation of TNF and LIF under normal and pathological conditions. The BBB consists mainly of specialized cerebral microvascular endothelial cells and constitutes a dynamic regulatory interface mediating the interactions of circulating cytokines with the CNS. The receptor-mediated transport system for LIF facilitates its restricted permeation from blood to brain and spinal cord in normal mice (23). Spinal cord injury causes upregulation of blood-spinal cord barrier uptake of LIF, so that the transport system functions at a higher level (18). The specific patterns and regulatory changes are also seen in studies of TNF transport (16;17;20–22;24). LPS has been shown to induce regulatory changes of other transport systems at the BBB level, including increased insulin influx (36) but decreased leptin influx (14). The results suggest that the BBB responds to LPS challenge by tight control of energy balance and cytokine signals.

Based on the evidence of receptor-mediated transport of LIF, upregulation of microvascular gp130 mRNA in the brain by LPS, and the regulatory changes of the BBB transporters specified above, we therefore formulated the hypothesis that LIF transport across the BBB is subject to regulation by LPS. As the effects of LPS may be mediated directly by activation of TLR4 or by stimulation of macrophage production of TNF and other proinflammatory cytokines, we also determined whether TNF plays a mediatory role. This was tested by analysis of BBB transport function in relation to the level of expression of cytokine receptors after LPS treatment, and by comparison with BBB transport in knockout mice deficient in TNF receptors, TNF itself, or the major signaling molecule NF κ B.

Materials and Methods

Homozygous TNF knockout and TNF double receptor (p55 and p75) knockout mice were studied along with their strain control B6.129SF2/J. Homozygous p50 NF κ B knockout mice were used with their control B6.129SP2/J mice. All of these were purchased from the Jackson Laboratory (Bar Harbor, ME). CD1/ICR outbred mice were purchased from Charles River Laboratories (Wilmington, MA). These male mice were studied at age 6–8 weeks, following protocols approved by the Institutional Animal Care and Use Committee. Recombinant mouse LIF was purchased from Chemicon (Temecula, CA), labeled with ¹²⁵I (Perkin Elmer, Waltham, MA) by the iodogen method, and purified by elution on columns of Sephadex G-10,

resulting in a specific activity of about 70 Ci/g, as described previously (18). The antibodies for LIFR, gp130, TNF receptor (TNFR)1, and TNFR2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The reagents for real-time PCR were purchased from Applied Biosystems (Foster City, CA). Unless specified, other chemicals were obtained from Sigma (St. Louis, MO).

LPS treatment

For mouse studies, LPS (5 mg/kg body weight, from *E. coli*, catalogue no. L6511, Sigma) was dissolved in pyrogen-free sterile normal saline (NS) at 5 mg/ml, and delivered to mice by intraperitoneal (ip) injection. The dosing was adopted from reports in the literature (6;28). The control group was injected with pyrogen-free NS in the same volume. A sample size of 8–10 mice/group was used for transport assays, and 4 – 6 mice/group were used for either RNA or protein measurement. The mice were observed at least twice daily for food intake, weight loss, and general malaise, and care was taken to avoid hypothermia. At 48 h after the single injection, mice were anesthetized for terminal studies (transport assay or tissue collection).

Multiple-time regression analysis

Groups of mice were anesthetized with ethyl carbamate (40 % aqueous solution) ip. ^{125}I -LIF (30,000 cpm/ μl) and sodium fluorescein (10 % solution) were dissolved in lactated Ringer's solution containing 1 % bovine serum albumin. A bolus of 100 μl of the mixed tracers was injected into the exposed left jugular vein at time 0. At 1 – 20 min (different mice for each time point), blood was collected from the right common carotid artery, and the mouse was decapitated immediately afterwards. The brain, excluding the pituitary, pineal gland, and hypothalamus, was collected and weighed. The radioactivity of ^{125}I -LIF in 50 μl of serum and brain was measured in a γ -counter (Wallac, Gaithersburg, MD). The endpoint was based on our previous results showing that most of the radioactivity in the brain represents intact ^{125}I -LIF at 20 min rather than degradation products (23). The brain/serum ratio of radioactivity was plotted against exposure time, the latter being the theoretical value of steady-state serum radioactivity as previously described (11). The regression correlation was determined with Prism GraphPad statistical software (San Diego, CA) to calculate the influx rate (slope of the linear regression) and the initial volume of distribution in the brain (the y-intercept of the regression line).

After measurement of the radioactivity, the brain and serum samples were dissolved in 1 and 0.2 ml of Solvable (Perkin-Elmer, Boston, MA), respectively, and digested for 40 h at 50 °C in the dark. The relative fluorescent intensity of 200 μl of the solution was measured in a fluorescent microplate reader (Spectramax Gemini, Molecular Devices, Sunnyvale, CA), with Solvable as the blank control. The excitation wavelength was 485 nm, the emission wavelength 538 nm, and the cutoff was 530 nm. The brain/serum ratio of fluorescence, corrected by brain weight for volume variation during digestion, was plotted against time of iv circulation. The difference between the LPS-treated and control group was compared with Prism GraphPad statistical software as mentioned above.

RNA extraction and real-time PCR

Microvessel-enriched fractions from the cerebral cortices of the mice were obtained by the capillary depletion method as described previously (19). Total RNA was extracted from the microvessels with an Absolutely RNA RT-PCR kit (Stratagene, La Jolla, CA). Reverse transcription and real-time PCR were performed with the one-step core PCR kit from Applied Biosystems (Foster City, CA). The primers and fluorescent probes for LIFR (gp190), the LIF co-receptor gp130, TNFR1 (p55), TNFR2 (p75), and the housekeeping gene GAPDH are listed in Table 1. Standard curves for the target genes were generated. The levels of expression of

cytokine receptors were normalized to that of GAPDH, and group means were compared by analysis of variance (ANOVA).

Western blotting

Microvessel-enriched fractions obtained by capillary depletion were lysed at 4 °C in a modified radioimmunoprecipitation (RIPA) buffer in the presence of a complete protease inhibitor cocktail, as described previously (39). Protein concentration in the supernatant was determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, IL). Forty µg of protein was electrophoresed, transferred to nitrocellulose membranes, and probed with primary antibodies against LIFR, gp130, receptor for ciliary neurotrophic factor (CNTF) CNTF-Rα, TNFR1, TNFR2, and the housekeeping gene β-actin (Santa Cruz Biotechnology), followed by horseradish peroxidase-conjugated secondary antibody. The signal was developed with an enhanced chemiluminescence kit (Pierce). The signal intensity was quantified with NIH Image J software and normalized to that of β-actin in the same samples. Group means are presented with their standard errors in the bar graphs, and significant changes were evaluated by ANOVA with SPSS software.

Results

1. LPS increases BBB permeability to LIF without affecting permeability to fluorescein

Two groups of CD1 mice were studied. The control mice injected ip with NS 48 h earlier showed an influx rate of 0.26 ± 0.05 µl/g-min and an initial volume of distribution of 15.15 ± 0.83 µl/g (n = 10/group). The LPS treated mice (5 mg/kg) had an influx rate of 0.69 ± 0.07 µl/g-min and initial volume of distribution of 17.98 ± 1.09 µl/g in the brain 48 h later (n = 8/group). The difference between these influx rates was significant ($p < 0.001$) (Fig. 1A). By contrast, there was no significant influx of sodium fluorescein from blood to brain in either group, despite a relatively high volume of distribution (Fig. 1B). This indicates that fluorescein is retained in the cerebral vasculature without crossing the BBB to reach brain parenchyma, and that LPS treatment did not cause a substantial disruption of the BBB at this time to increase the paracellular permeation of fluorescein.

2. LPS increases gp130 expression of both mRNA and protein to a greater extent than LIFR expression

In microvessels from mice treated with LPS 48 h earlier, there was a significant increase in the level of mRNA for gp130 ($p < 0.05$). The increase was not significant for LIFR (Fig. 2A). The increase in gp130 mRNA expression was paralleled by elevated protein signals shown by western blotting analysis ($p < 0.01$) (Fig 2B and 2C). Thus, the challenge induced by LPS caused transcriptional activation of gp130.

LIF, CNTF, and cardiotrophin-1 are closely related members of the neuropoietic cytokine family. They share the receptors LIFR and gp130 to induce receptor phosphorylation by associated JAK tyrosine kinases and STAT3 activation. CNTF requires an additional subunit - CNTF-Rα - for receptor binding and activation (5). To determine whether the LPS induces specific changes of LIF receptors, we also measured the level of expression of CNTF-Rα. There was no significant difference of signal intensity of CNTF-Rα in the cerebral microvessels obtained after NS or LPS treatment (n = 5/group) (Fig. 2D).

3. LPS-induces a concurrent increase of the receptors for TNF (TNFR1 and TNFR2)

In enriched microvessels of LPS-treated mice, the mRNA for both TNFR1 and TNFR2 was significantly increased (Fig. 3A). Western blot showed that the proteins of both TNF receptors

were also increased after LPS treatment (Fig. 3B). Densitometry analysis of the signals indicated that the increase was significant ($p < 0.05$) (Fig. 3C).

4. Lack of increase of gp130 in TNF receptor knockout mice

The significant ($p < 0.001$) weight loss occurring 48 h after injection of LPS in the TNF ligand knockout mice and TNF receptor knockout mice was similar to that seen in the control strain, indicating that all groups of mice mounted an inflammatory response (Fig. 4A). Just as the increase in response to LPS was not significant for LIFR mRNA in the experiment discussed in the preceding paragraph and shown in figure 2A, a similar experiment in TNF knockout mice and TNF receptor knockout mice also did not show regulatory changes of LIFR mRNA (Fig. 4B). Strikingly, however, the significant upregulation by LPS of gp130 mRNA in cerebral microvessels from the control strain did not occur in TNF ligand knockout and TNF receptor knockout mice (Fig. 4C). The level of protein expression showed similar changes, with LPS causing a significant increase of the gp130 signal in B6 mice, which was not seen in the TNF (TKO) or TNF receptor (RKO) knockout mice (Fig. 4D–E).

5. Reduced gp130 upregulation in the p50 knockout mice indicates that the effects of LPS involve NF κ B

Expression of gp130 mRNA in the control strain tended to increase ($p = 0.06$), whereas the non-significant change of gp130 mRNA in response to LPS treatment in the NF κ B-deficient mice was slightly in the opposite direction (Fig. 5A). The results were somewhat similar for LIFR, with LPS non-significantly increasing mRNA expression in the controls ($p = 0.13$), but non-significantly decreasing it in the knockouts ($p = 0.14$). The lack of robust significant changes, particularly in LIFR mRNA expression, may have been influenced by the small sample size ($n = 2$ /saline treatment, and $n = 3$ /LPS treatment). There might also be strain differences in the response to LPS, as the B6.129SF/J mice (background control for TNF ligand and receptor knockouts) were more responsive than B6.129SP/J mice (background control for p50 NF κ B subunit knockout). Regardless, LPS was no longer effective in the p50 NF κ B subunit knockout mice.

Discussion

We chose to study LIF transport after LPS injection based on compelling evidence from the literature indicating that LIF is a marker for the severity of septic shock and plays a pivotal role in CNS inflammation and autoimmune diseases. In the spinal cord, LIF is a potent proinflammatory cytokine, as shown by overexpression (12) and knockout studies in a seizure model (9). LIF promotes oligodendrocyte survival after spinal cord injury (13) and limits demyelination and oligodendrocyte loss in mouse models of experimental autoimmune encephalomyelitis (3;4). As inflammation and autoimmunity can also be neuroprotective (8; 27), the fine balance necessary to achieve an adequate inflammatory response in the CNS without exacerbation of inflammation and cell death can be readily achieved by regulatory changes of BBB transport.

LPS binds to the CD14/TLR4/MD2 receptor complex, induces transcriptional activation, and eventually stimulates production of proinflammatory cytokines. The proinflammatory cytokine LIF appears to have a beneficiary role in counteracting LPS-induced endotoxic shock (35), suggesting its potential to provide negative feedback in exaggerated host defense. At the BBB level, we show here that LPS can cause a significant increase in the permeability of LIF from blood to brain. Thus, the CNS responds to LPS challenge by increasing the availability of blood-borne LIF, which has a relatively low basal permeation in normal mice (23).

LPS has been reported to increase the permeability of the BBB to radioactively labeled albumin. This was observed after three consecutive doses of LPS (3 mg/kg in lactated Ringer's solution) ip, 8 h apart, before examination of CD1 mice at 24 h. However, a single injection of 3 mg/kg did not induce an increase of albumin uptake 8 h later (15), and this is consistent with our observation that the paracellular BBB permeability to sodium fluorescein was not increased 48 h after a single injection of LPS. The decreased BBB permeation of pituitary adenylate cyclase activating peptide (15) and leptin (14) after LPS, but increased BBB permeation of insulin (36), also supports the specificity of the regulatory changes.

In our study, a fluorescent tracer, which is much smaller than albumin, was used to detect any subtle alterations of the BBB. Fluorescein is a molecule of only 376 Da that serves as a sensitive marker for paracellular permeability. It also is a substrate for the efflux transporters multidrug resistance protein (MRP)-2 and organic anion transporter (OAT)-3 (29). In rats with diabetes mellitus induced by streptozotocin, the permeation of fluorescein is decreased after 4 min of in-situ brain perfusion, probably related to activation of efflux transporters (7). Although NF κ B mediates upregulation of multidrug resistance protein 1 (MDR1) by TNF (37;38) and LPS (Yu et al, unpublished observations), LPS did not increase fluorescein influx in our study. Thus, LIF enters the brain in mice treated with LPS by specific upregulation of its transport system at the BBB level.

To further determine the mechanisms of upregulation of LIF transport by LPS, we first measured the level of expression of LIF receptors in enriched cerebral microvessels. LPS mainly increased the expression of gp130, both at the mRNA and protein levels. This indicates a transcriptional activation. The findings contrast with the lack of significant increase of LIFR (gp190) in the same samples. Thus, LPS differentially affected the expression of the two receptors for LIF. Since CNTF uses an additional receptor subunit CNTF-R α in addition to LIFR and gp130, we also determined the level of CNTF-R α protein expression. There was no significant change in the cerebral microvascular CNTF-R α caused by LPS treatment shown by western blotting. This further indicates the selectivity of gp130 upregulation.

The correlation of elevated gp130 and LIF transport suggests that gp130 in cerebral microvessels mediates the increased transport of LIF across the BBB. This in-vivo observation differs from our in-vitro results in RBE4 cerebral microvessel endothelial cells, in which binding and endocytosis of LIF were reduced after TNF treatment accompanied by decreased LIFR and increased gp130 (40;41). Since the cell studies involved stimulation with TNF, rather than LPS, and a shorter duration of treatment than received by the mice, the discrepancy probably reflects the complexity of dynamic changes in-vivo. This prompted us to examine the role of the TNF system in LPS-induced upregulation of LIF transport.

In cerebral microvessels from CD1 mice, LPS induced both TNFR1 and TNFR2 mRNA and protein 48 h after treatment. To determine whether the TNF system plays a role in mediating LPS-enhanced LIF transport, we used knockout mice lacking either functional TNF receptors or TNF itself, and compared the induction of LIF receptors with the respective strain background control (B6.129SF2/J). Double TNF receptor knockout mice have elevated serum concentrations of TNF and increased resistance to LPS-induced lethality, an effect mainly conferred by the p55 receptor (TNFR1) (26). The p75 receptor TNFR2 plays an important role in vasculopathy, as human transmembrane TNF/p75TNFR transgenic mice show prominent cerebral endothelial activation, meningeal inflammation, and vessel fibrosis with vasculitis eventually leading to vessel occlusion and multifocal infarct (1). TNF knockout mice show a deficiency in humoral immune responses, with an absence of splenic primary B cell follicles and inadequate formation of follicular dendritic cell networks and germinal centers. These mice are resistant to the systemic toxicity of LPS at the usual doses (25).

Our LPS treatment regimen was effective in the TNF ligand and TNF receptor knockout mice as well as in the control strain, with reduction of body weight and selective induction of cytokine receptors. However, the significant increase of gp130 mRNA and protein expression was no longer present when the knockout mice were treated with LPS. This indicates a critical role of TNF in mediating the increased transport of LIF after LPS treatment. The opposing changes of LIF transport in the wildtype mice treated with LPS and RBE4 cells treated with TNF, therefore, may suggest that the regulation of LIF in LPS-treated mice also involves secondary mediators in addition to TNF.

Both LPS and TNF can activate NF κ B as well as other transcription factors, and they use non-transcriptional mechanisms to induce cellular effects. Activation of NF κ B requires dimerization of the p50 and p65 subunits, which are dissociated from the inhibitory I κ B subunit, and nuclear translocation and binding to responsive gene promoters. Mice deficient in the p50 subunit, therefore, do not mount effective NF κ B activation. With p50 knockout mice, we showed that LPS failed to induce transcriptional activation of gp130 and elevate its protein level. Therefore, LPS-induced upregulation of the gp130 receptor requires NF κ B activation.

In summary, LPS caused a significant increase of basal LIF transport across the BBB 48 h after the challenge in mice. This upregulation was mainly mediated by gp130 and modified by TNF. It was absent in TNF ligand knockout mice and in TNF receptor knockout mice. The effects of LPS and its subsequent induction of TNF require transcriptional activation by NF κ B. Thus, neuroinflammation leads to upregulation of LIF transport across the BBB by modification of cerebral endothelial signaling.

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List of abbreviations

BBB	blood-brain barrier
CNTF	ciliary neurotrophic factor
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
IL-6	interleukin-6
LPS	lipopolysaccharide
LIF	leukemia inhibitor factor
LIFR	leukemia inhibitor factor receptor (gp190)
NFκB	nuclear factor κ B
TLR4	

toll-like receptor 4

TNF

tumor necrosis factor α

TNFR1

tumor necrosis factor receptor 1 (p55 or p60)

TNFR2

tumor necrosis factor receptor 2 (p75 or p80)

TKO

tumor necrosis factor (ligand) knockout mouse

RKO

tumor necrosis factor receptor knockout mouse

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Fig. 1. The influx rate of ^{125}I -LIF and fluorescein from blood to brain in mice treated with LPS (5mg/kg) or saline (control) for 48 h. LPS treatment significantly increased the entry of ^{125}I -LIF from blood to brain (A) without affecting the entry of fluorescein (B).

Fig. 2.

Microvascular expression of LIF receptors 48 h after LPS treatment. (A) The increase of mRNA expression was significant for gp130 but not for LIFR. $n = 6/\text{group}$. (B) Western blotting showed an increased signal for gp130, but not for LIFR or the internal control β -actin, in microvessels obtained from the brain of LPS-treated mice. $n = 7/\text{group}$. (C) Densitometric analysis of western blotting data indicated a significant increase of gp130, but not LIFR. *: $p < 0.05$; **: $p < 0.01$. (D) Similarly, there was no change in the signal intensity of CNTF-R α by LPS treatment ($n = 5/\text{group}$).

Fig. 3.

Induction of TNF receptors in cerebral microvessels from mice treated with LPS 48 h earlier. (A) The mRNA for both TNFR1 and TNFR2 was significantly increased in the LPS group compared with the normal saline (NS) control. $n = 6/\text{group}$. (B) Western blotting showed that the signals for TNFR1 and TNFR2 were increased, whereas that for β -actin was unchanged. $n = 7/\text{group}$. (C) Densitometric analysis of western blotting data, indicating a significant increase of TNFR1 and TNFR2. *: $p < 0.05$; **: $p < 0.01$.

Fig. 4.

Differential microvascular responses to LPS treatment in the induction of LIF receptors in the TNF ligand knockout (TKO, $n = 5/\text{group}$), TNF receptor double knockout (RKO, $n = 4/\text{group}$), and background strain control mice ($n = 7/\text{group}$). All three types of mice showed significant weight reduction in response to LPS (A). The mRNA for LIFR showed no significant changes (B). The mRNA for gp130 was increased significantly only in the B6 mice, but not in the TKO or RKO mice (C). Concurrently, there was an increased signal of gp130 in the B6 mice only, shown by western blot analysis (D). The significant increase was also shown by densitometric analysis (E). *: $p < 0.05$; **: $p < 0.01$.

Fig. 5.

Real-time RT-PCR of cerebral microvessels obtained from the p50 NF κ B subunit knockout mice, to test the expression of LIF receptors in response to LPS treatment. In the control strain, LPS treatment (n = 2) tended (p = 0.06) to increase the mRNA for gp130 (A) but not for LIFR (B). In the p50 knockout mice, LPS failed to induce an increase of the receptor mRNAs.

Table 1

Real-time PCR primers and probes

	Primers (5' → 3')	6-FAM probe
LIFR (gp190)	F: AGTGGCTGTGGCTGTCATTGT R: TGGGTAGAATGTTTCCTTAATCCATT	CGAGCATCCTTTGCTATCGGAAGC
gp130	F: CCAGCAACGAGGAGAATGAGT R: TGTGCACCACAGTGGAGTACTG	TCAGAGCACCGCCAGCACGG
TNFR1	F: CCGACTTGGTGTACATAGCTTTTCT R: GGTACATCTCCCTGCCACTCA	CCGCCGAGGACTGCCTGAGC
TNFR2	F: GGATGAGCAGGTCCCTTCT R: CTCTGCAGTGTCTCTGTAGTCTCACA	CAGGAGGAGTGTCCGTCTCAGTCCCC
GAPDH	F: GAAGGTGAAGGTCGGAGTC R: GAAGATGGTGATGGGATTTC	CAAGCTTCCCGTTCTCAGCC