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Endogenous leukemia inhibitory factor attenuates endotoxin response

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Leukemia inhibitory factor (LIF) is induced in inflammation and likely plays a regulatory role. Using LIF-deficient mice (LIF−/−), we report here that endogenous LIF has a protective role in endotoxic shock and host defence. LIF−/− mice have heightened sensitivity to LPS in a LPS/D-galactosamine (D-Gal) sensitization model compared to wild-type mice (LIF+/+), enhanced thrombocytopenia and leukopenia, with increased hepatic necrosis, neutrophil sequestration in the lung and accelerated mortality. These findings correlated with 10-fold higher tumour necrosis factor-α (TNFα) and interleukin-6 (IL-6) serum levels and reduced IL-10 production in LIF−/− mice in response to LPS. Therefore, endogenous LIF attenuates the endotoxic shock response, enhances the expression of basal acute phase proteins and IL-10 production, which downregulates TNFα synthesis and release and thereby confers partial protection to endotoxemia.
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Leukaemia inhibitory factor (LIF) is a secreted cytokine with pleiotropic functions, which include the inhibition of adipogenesis, neuronal and stem cell survival, induction of the acute-phase response proteins and embryo implantation.[1] LIF, like tumour necrosis factor alpha (TNFα), interleukin-1 (IL-1) and IL-6, is involved in mediating aspects of the inflammatory response such as stimulation of acute-phase protein (APP) synthesis, induction of cachexia and inhibition of lipoprotein lipase (LPL) activity[2] and hypothalamic pituitary adrenal axis responses.[3] A partial functional redundancy of LIF with IL-6, IL-11, ciliary neutrotrophic factor (CNTF) and oncostatin M (OSM) is due to the fact that all these ligands utilize the common receptor subunit gp130, together with their specific receptors.[4–6] LIF can be induced by TNFα, IL-1 and LPS, and is expressed by a variety of cell types *in vitro*, including fibroblasts, activated T cells, monocytes, macrophages, bone marrow stromal cells, astrocytes and endothelial cells, as well as by a number of tissues in adults.[4,7] Clinical studies demonstrated that serum concentrations of LIF are elevated in patients with Gram-negative and meningococcal septic shock[8] and that the levels correlated with the severity of disease.[9] In murine endotoxic shock models LIF is induced after intraperitoneal administration of LPS 10, where it can be detected in circulation after 1–3 h.[10,11] LIF given prior to a lethal challenge to LPS[12] or to live *Escherichia coli*[11] provided nearly complete resistance to these agents. Furthermore, LIF coinjected with LPS into the rat trachea decreased LPS neutrophil recruitment.[13] A recent study demonstrated that LIF has an anti-inflammatory role in cutaneous inflammation using a LIF adenoviral vector, while inflammation was increased in LIF-deficient mice.[14] In contrast, Block *et al*[15] reported that neutralization of LIF by the administration of rabbit IgG prevented LPS-induced lethality and cytokine release in mice.

Here, we used LIF-deficient mice[16] to elucidate the role of LIF in the cytokine network regulating the response to endotoxic shock. Endotoxic shock was elicited by LPS in the presence or absence of D-galactosamine (D-Gal) sensitization.[7,17–19] Our results reveal that LIF-deficient mice are more sensitive to LPS-mediated shock. This is associated with a diminution in the basal expression of APP, reduced IL-10 production together with prolonged elevation of TNFα and IL-6, suggesting that LIF may have an important role in mitigating the endotoxic shock response by inhibition of TNFα and IL-6 synthesis and release.

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Materials and methods

Animals

In all, 10- to 20-week-old mice on a Balb/c background (both LIF+/+ and LIF−/−) were produced by the team of Dr Stewart and in few additional and confirmatory experiments from Dr Brulet[16,20] and maintained in a barrier animal facility. The animals were housed in 12 h light/dark cycles and received food and water *ad libitum*.

Reagents

LPS from *E. coli* (serotype 0111:B4) and D-Gal were purchased from Sigma (St Louis, MO, USA) and dissolved in pyrogen-free sterile saline (Abbott Lab., IL, USA). Recombinant LIF was purchased from EMD Biosciences, San Diego, CA, USA.

Experimental Protocol

Mice weighing about 25 g were injected intraperitoneally with either LPS alone (1 and 10 μg) or LPS (100, 10 and 1 ng) in combination with D-Gal (20 mg) in saline. Blood was collected from the retro-orbital sinus into EDTA-coated tubes for haematology or into heparinized tubes for blood plasma 1–2 days prior to LPS administration for baseline values and at 1, 6, and 24 h after LPS challenge and plasma was frozen at −20°C until further processing. Body weight, clinical signs and mortality were recorded at regular intervals. Plasma aminotransferases were measured on a Cobas Fara Analyzer (Hoffmann-La Roche Inc.). Further, mice were injected recombinant LIF 6 and 1 h by the subcutaneous route (s.c.) prior to LPS/D-Gal challenge to test whether exogenous LIF could confer protection and/or reverse toxicity in and LIF−/− mice.

Primary Macrophage Cultures

Murine bone marrow cells were isolated from femurs from LIF+/+ and LIF−/− mice and cultivated (10^6/ml) for 7 days in Dulbecco's minimal essential medium (DMEM) supplemented with 2 mM L-glutamine and 2×10^{-5} M β-mercaptoethanol, 20% horse serum and 30% L929 cell-conditioned medium (as source of M-CSF) yielding a cell culture containing more than 95% macrophages.[21] The bone marrow-derived macrophages (BMDM) were plated in 96-well microculture plates at a density of 10^5 cells/well in DMEM supplemented with 2 mM L-glutamine and 2×10^{-5} M β-mercaptoethanol and stimulated with 100 ng/ml LPS (*E. coli*, serotype O111:B4, Sigma). After 6 to 24 h of stimulation, the supernatants were harvested and analysed immediately or stored at −20°C until further use.

Cytokine Determination

Cell supernatant and plasma were assayed for cytokine content using commercially available ELISA reagents for TNFα, IL-6 and IL-10 as described before (R&D Systems, Abingdon, UK and BD Pharmingen, San Diego, CA, USA). Preliminary experiments showed TNFα peak plasma levels at 1 h and IL-6 levels peak at 6 h. Thereafter, all TNFα and IL-6 measurements in the D-Gal/LPS model were performed on plasma from controls, 1 and 6 h after treatment.

Determination of APP

Serum amyloid A protein (SAA) was measured by ELISA according to the manufacturer's instructions (Cytoscreen Immunoassay Kit, Biosource International, Camarillo, CA, USA). Serum amyloid P protein (SAP) was measured by a sandwich ELISA using sh-α-mSAP as coating antibody, rb-α-mSAP as secondary antibody (both Calbiochem, San Diego, CA, USA) and gt-α-rblgG-alkaline-phosphatase (Sigma) as detecting antibody. Concentrations were calculated using mSAP-standard (Calbiochem, San Diego, CA, USA) as a reference.

Northern Blot Analysis of Acute-Phase mRNAs

Total hepatic RNA was isolated by the guanidine isothiocyanate method[22] from livers of mice injected with D-Gal/LPS at 0, 1, 2, 9 and 24 h. Northern blot analysis and hybridization with labelled cDNAs for serum amyloid A2 (SAA2) and SAA3, alpha-1-acid glycoprotein (AGP), haptoglobin (Hp), haemopexin (Hpx) and GAPDH was performed as described.[17] Signals were quantified by phosphorimager analysis and normalized against the internal control GAPDH.

Histological Investigation

The Lung, liver and spleen sections were fixed in 10% neutral buffered formalin, embedded in paraffin blocks, sectioned at 4 μm, and stained with haematoxylin and eosin as described before.[19] Sections from the liver and lungs of five mice per group were analysed.

Statistical Analysis

Analysis was performed using Student's *t*-tests, Mann–Whitney test and values of $P<0.05$ were considered significant. Survival were analysed by Kaplan–Meier test. Each experiment was repeated at least once to ensure reproducibility (see text and figures).

Results

Enhanced Endotoxic Shock in D-Gal-Sensitized LIF−/− Mice

First, the effect of LPS (1 μg) on the circulating blood cells was investigated. At 6 h, a significant leukopenia and thrombocytopenia was observed, which was more pronounced in the absence of LIF (Table 1).

The sensitivity of LIF-deficient mice to lethal endotoxic shock was then determined at three LPS doses (100, 10 and 1 ng) in D-Gal-sensitized mice. This well-established hypersensitivity model makes use of the sensitising effect of D-Gal increasing the lethal effect of LPS and greatly potentiating liver injury.[17,23]

LIF−/− animals displayed more pronounced clinical symptoms of shock with huddled posture, piloerection, shivering, diarrhoea and body weight loss (Figure 1a), and succumbed between 6 and 12 h after administration, while wild-type mice showed a higher resistance to endotoxin and succumbed later (Figure 1b). The levels of alanine (ALT) and aspartate (AST) transaminases, enzyme markers of hepatocellular damage, were significantly increased in LIF−/− mice at 6 and 24 h (Table 2).

Microscopic examination of the livers revealed vascular congestion, haemorrhage, hepatocellular vacuolization, fragmentation, pyknosis and necrosis 6 h after LPS injection, which was more prominent in LIF−/− mice than in the wild-type controls (Figure 2a). The lungs, which is another target organ of endotoxic shock, showed increased recruitment of neutrophils in LIF−/− mice (Figure 2b), which was confirmed by analysis of myeloperoxidase expression in the lung, which was significantly enhanced in the absence of LIF (data not shown).

Lastly, prior administration of recombinant LIF by the subcutaneous route (two injections at 1 μg) attenuated LPS-induced leukopenia in LIF−/− mice (Table 3). Furthermore, the LPS–D-Gal induced increase of hepatic enzymes was also ameliorated in LIF−/− and wild-type mice (Table 3). These data suggest that exogenous LIF can abrogate the enhanced LPS toxicity in the absence of LIF.

In summary, LPS had an augmented toxic effect on the haematological parameters, the lung and liver in the absence of LIF, which could be attenuated by exogenous LIF administration.

Table 1 Enhanced thrombocytopenia and leukopenia in LIF−/− mice upon LPS injection

	LPS (μg)	*Leukocytes*	*Lymphocytes*	*Thrombocytes*
LIF−/−	0	12.7±1.8	10.8±1.4	467±38
LIF+/+	0	13.2±2.1	11.4±1.1	458±31
LIF−/−	1	4.2±0.5¶§	2.9±0.3¶	126±21¶§
LIF+/+	1	8.6±0.7¶	3.5±0.4¶	291±18¶

Blood was taken at 6 h after LPS or saline injection. Values are mean±s.d. and represent 1000 cells/μl blood ($n=4$–6 mice per group).
¶$P<0.05$ control *vs* LPS administration, §$P<0.05$ in LPS-treated mice.

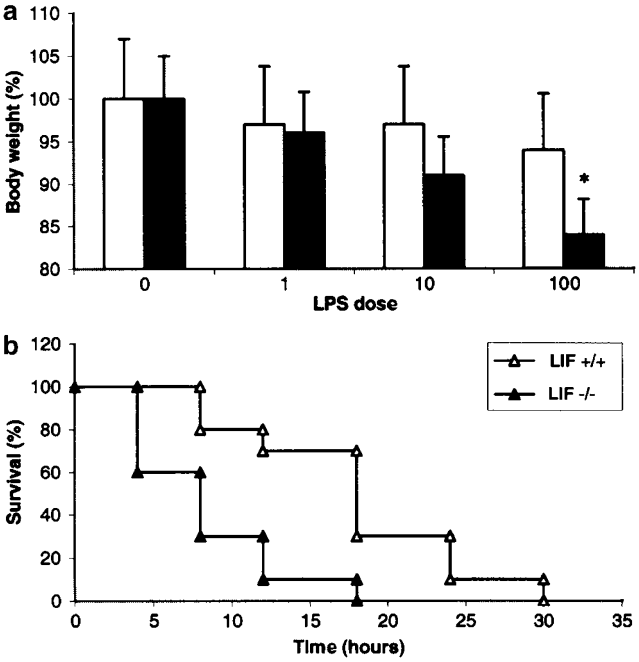

Figure 1 Increased body weight loss (**a**) and mortality (**b**) after LPS injection in D-Gal sensitized LIF−/− mice (closed bars or triangles) and LIF+/+ mice (open bars or triangles). Mice were injected with LPS (1–100 ng) together with D-Gal as described in Materials and methods; body weight was recorded at 12 h, expressed as percentage of initial body weight. The relative body weights of the LIF−/− mice at the 100 ng LPS dose were significantly lower than LIF−/− mice ($P<0.05$). Survival was recorded over 30 h after LPS (100 ng) and was significantly higher in LIF+/+ mice ($n=10$ mice).

Table 2 ALT and AST plasma levels at 6 and 12 h upon LPS–D-Gal injection in LIF+/+ and LIF−/− mice

	LPS (ng)	*6 h*		*12 h*	
		LIF−/−	*LIF+/+*	*LIF−/−*	*LIF+/+*
ALT (IU/ml)	1	56±4	54±5	368±84§	102±14
	10	91±10	84±10	1499±189§	487±87
	100	1037±132§	898±121	—	—
AST (IU/ml)	1	118±12	105±8	868±114§	254±45
	10	134±8	118±11	9123±234§	436±87
	100	1091±91§	931±76	—	—

Mice received 20 mg D-Gal and LPS at 1 to 100 ng per mouse. Values are mean±s.d. §$P<0.05$ between experimental groups at given LPS dose ($n=4$–6 mice per group).

The enhanced toxicity of LPS in LIF−/− mice might be due to increased proinflammatory cytokine production and altered acute-phase response.

Increased Endotoxin-Induced TNFα and IL-6 Serum Levels in LIF−/− Mice

We tested the possibility that cytokine secretion following LPS challenge was enhanced in the absence of LIF. Therefore, the serum concentrations of TNFα and IL-6 were determined after LPS

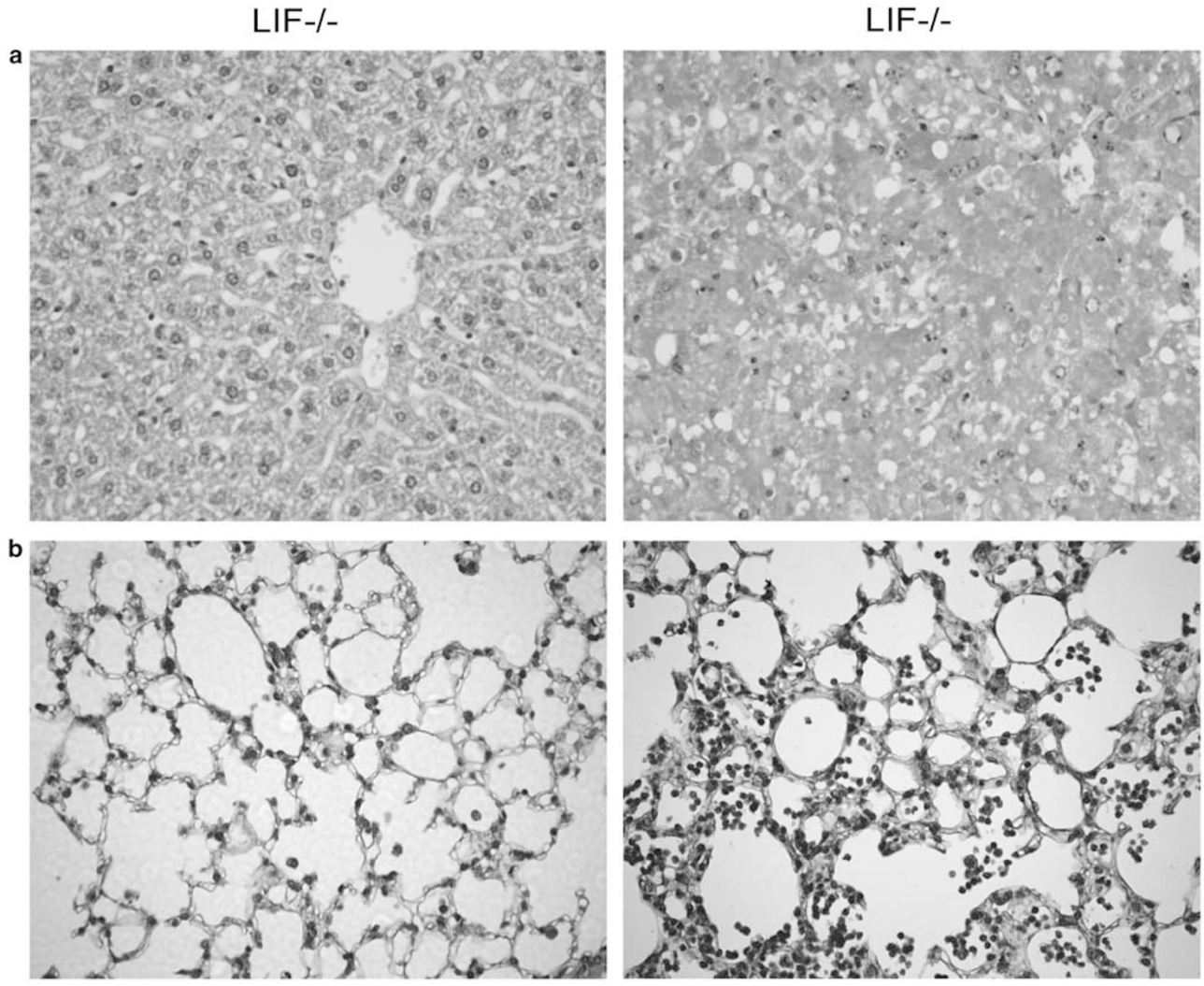

Figure 2 Enhanced liver damage and neutrophil recruitment into the lung induced by LPS injection in D-Gal-sensitized LIF−/− mice. (**a**) Hepatocellular necrosis induced by LPS (100 ng) injection in D-Gal-sensitized LIF−/− mice as compared to LIF+/+ mice. Representative micrograph showing distinct necrosis, pyknosis, karyorrhexis and vascular congestion in LIF−/− mice at 6 h after LPS–D-Gal injection, which was less pronounced in LIF+/+ mice ($n=6$ mice). (**b**) Augmented neutrophils recruitment induced by LPS (100 ng) injection in the lung of LIF−/− mice as compared to LIF+/+ mice. Haematoxylin and eosin, ×280 magnification ($n=6$ mice).

Table 3 Exogenous LIF attenuates leukopenia and liver damage in LIF+/+ and LIF−/− mice

LPS (100 ng)	*LPS*		*LPS and LIF (1 μg)*	
	LIF−/−	*LIF+/+*	*LIF−/−*	*LIF+/+*
Leukocytes	5.4±0.6§	8.2±0.9§	9.7±1.6	10.6±1.9
ALT (IU/ml)	863±91§	498±121§	175±64	170±92
AST (IU/ml)	851±91§	460±76§	163±58	124±76

Mice received 20 mg D-Gal and LPS at 100 ng per mouse, LIF (1 μg) was given 6 and 1 h before s.c. Mice were bled at 6 h for haematological and serum chemistry analysis. Values are mean±s.d. §$P<0.05$ between the groups receiving LPS *vs* LPS/LIF ($n=4$–6 mice per group).

administration (10 μg). In both LIF−/− and LIF+/+ mice, TNFα and IL-6 serum concentrations peaked at 1 and at 3 h, respectively. LIF-deficiency resulted in significant higher serum TNFα levels, which persisted for up to 6 h after LPS injection (Figure 3a). In contrast, wild-type mice had no detectable TNFα at 3 h. Similar findings were obtained with IL-6 showing higher and protracted IL-6 serum levels in LIF−/− mice than LIF+/+ mice (Figure 3b). We further tested the effect of the LPS dose in D-Gal-sensitized mice. TNFα serum level at 1 h was significantly elevated already at the 10 ng LPS dose, while IL-6 levels at 3 h were only induced at the 100ng dose (data not shown).

Therefore, endotoxin-induced TNFα and IL-6 serum levels were significantly increased and may have a protracted course in LIF−/− mice.

Decreased Endotoxin-Induced IL-10 Production in LIF−/− Mice

IL-10 is induced by LPS in macrophages/monocytes *in vitro* and *in vivo* in mice[24,25] and in septicaemia.[26]

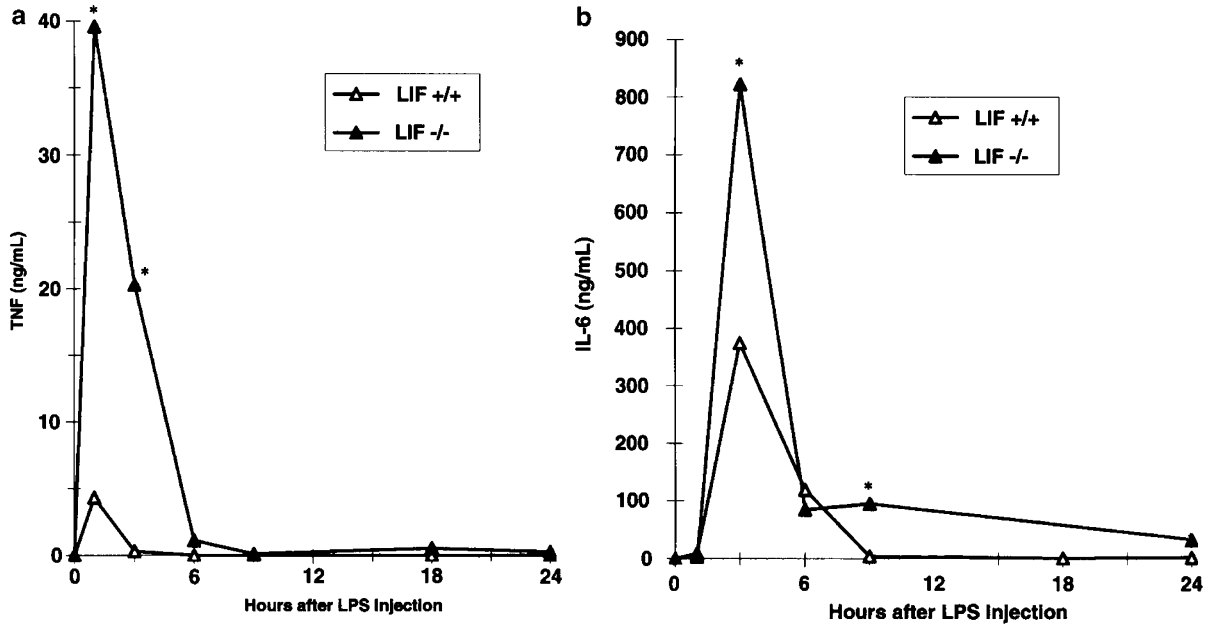

Figure 3 Time dependent changes of TNFα (**a**) and IL-6 (**b**) serum levels in LIF−/− mice upon LPS injection (10 μg). Peak TNFα and IL-6 serum levels at 1 and 3 h, respectively, were significantly higher in LIF−/− mice (black triangle) than in LIF+/+ mice (open triangle, $*P<0.05$). Mean values±s.d. are given ($n=5$ mice).

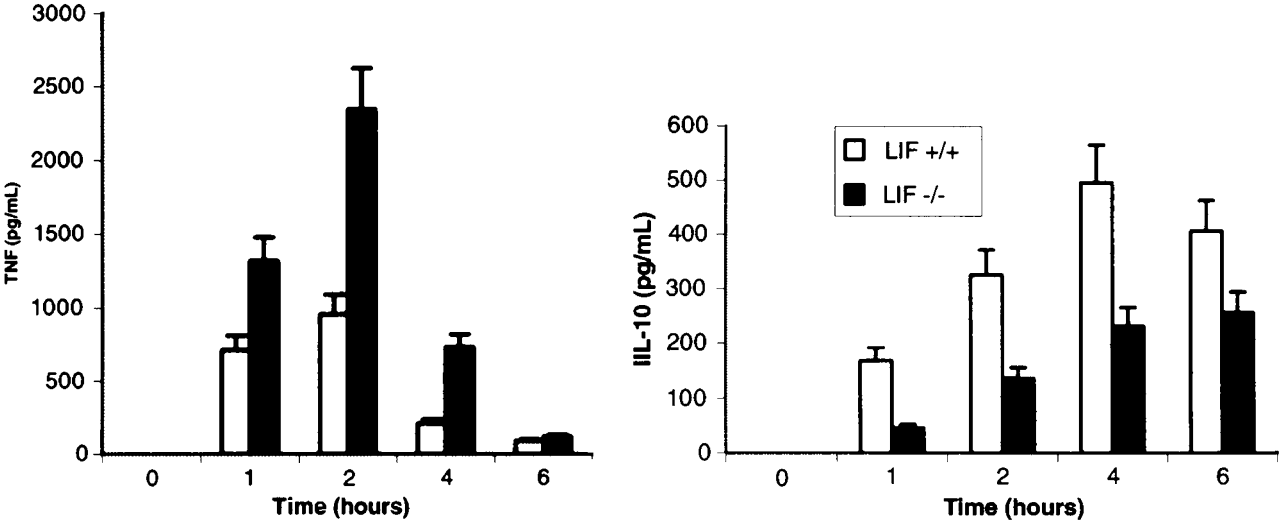

Figure 4 Increased TNF and reduced IL-10 production in LPS stimulated BMDM from LIF−/− mice. BMDM from LIF−/− mice (black bars) than in LIF+/+ mice (open bars) were stimulated with 100 ng/ml LPS and the cell supernatant was assessed for TNF and IL-10 at 1, 2, 4 and 6 h by ELISA. Mean values±s.d. are given, $*P<0.05$. Representative results from three independent experiments.

There is evidence that IL-10 downregulates TNFα production.[25,27] Therefore, we asked whether endogenous LIF affects IL-10 production. First, we tested TNFα and IL-10 production by bone marrow-derived macrophages. LPS-induced activation of macrophages in the absence of LIF resulted in enhanced production of TNFα, and diminished IL-10 secretion as compared to wild-type macrophages (Figure 4). Then, we tested the effect of LPS administration on IL-10 serum levels *in vivo*. In accordance with the effect on macrophages, the IL-10 levels were significantly reduced in LIF−/− mice upon LPS injection (Figure 5).

Therefore, the data suggest that in the absence of endogenous LIF LPS-induced TNFα production was augmented, which may be related to the diminished production of IL-10, a known regulator of TNFα production.

Decreased Basal and Enhanced LPS-Induced APP in the Absence of LIF

The acute-phase response has been shown to be protective in infection and endotoxic shock. The sensitizing effect of the LPS/D-Gal-model is likely

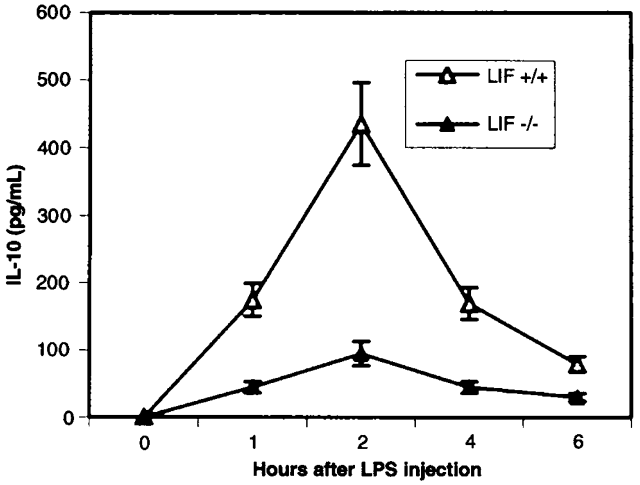

Figure 5 Decreased IL-10 plasma levels upon LPS injection in the absence of LIF LPS (10 μg) was injected i.p. in LIF+/+ and LIF−/− mice and serum was taken after LPS injection and serum was analysed by ELISA for IL-10 levels. Mean values ± s.d. are given, $^*P<0.05$ ($n=6$–8 mice).

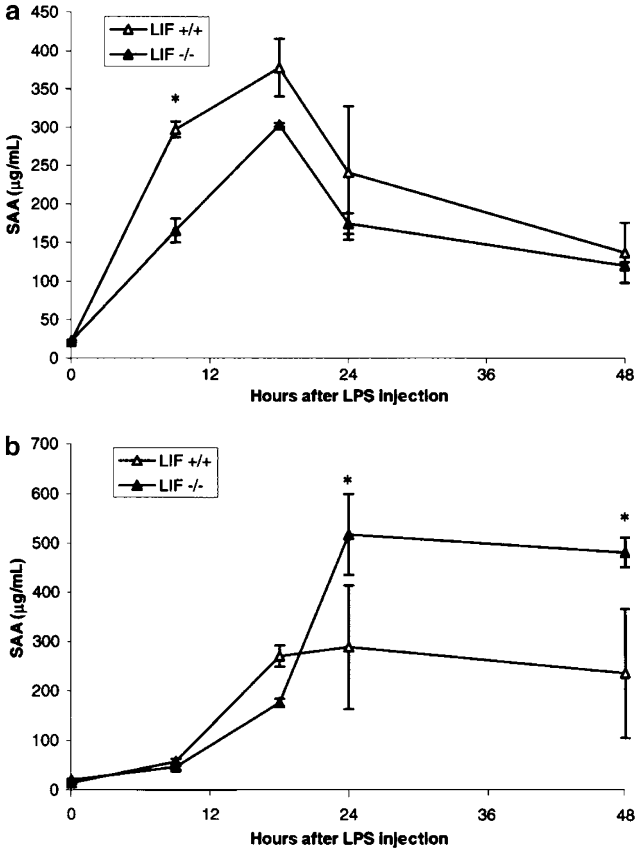

Figure 6 Low basal, but augmented SAA and SAP serum levels in LIF−/− mice upon LPS (10 μg) injection. SAA (**a**) and SAP (**B**) serum levels were determined over 2 days by ELISA in LIF−/− mice (closed triangle) and LIF+/+ mice (open triangle) as described under Materials and methods. SAA levels were higher in LIF−/− mice over 24 h upon LPS injection, while SAP levels were slightly delayed at 18 h, but augmented at 24 and 48 h in LIF−/− mice. The data were compared with the nonparametric Mann–Whitney test ($^*P<0.05$). Mean values ± s.d. are given ($n=5$ mice).

due to an inhibition of acute-phase reactants in the liver by a global hepatic synthesis inhibitor 23. The two major APP in mice, SAA and SAP were measured before and after LPS administration. The LIF−/− mice exhibited slightly lower basal serum levels of both proteins, which were, however, not significantly different from LIF+/+ mice. Upon LPS injection, SAA was rapidly induced, and the serum concentrations were initially significantly higher in LIF−/− mice (Figure 6a). SAP levels were initially lower in LIF−/− mice, but significantly superior at 24 and 48 h to those of LIF+/+ mice, which could be due to the sustained and elevated IL-6 levels (Figure 6b). It has been previously shown that cytokine-induced changes seen of APP secretion are in part reflected in mRNA expression. Northern blot analysis of RNA levels of SAA2, SAA3, Hp, AGP and HPX in the liver revealed a distinctive lower basal mRNA level in LIF−/− mice (Figure 7). However, mRNA levels for all five genes were induced by LPS to a similar extent in both types of mice except for heightened SAA3 levels at 2 and 9 h postinjection in LIF−/− mice (see Figure 7 and Table 4 for the quantification by densitometry). Therefore, steady-state transcripts and expression of APP, but not LPS-induced transcription or expression was lower, which might explain in part the heightened sensitivity of the LIF−/− mice.

Discussion

Our data demonstrate a critical protective role of endogenous LIF in endotoxin-induced pathology. Endotoxin injection into D-Gal-sensitized LIF−/− mice resulted in enhanced signs of shock, including a huddled posture, piloerection, shivering, diarrhoea, body weight loss and death. Death was preceded by leukopenia, and thrombocytopenia, enhanced neutrophil recruitment into the lung and hepatocellular damage. Hepatotoxicity of LIF−/− mice correlated with a 10-fold increased and protracted TNFα and IL-6 serum levels, reduced

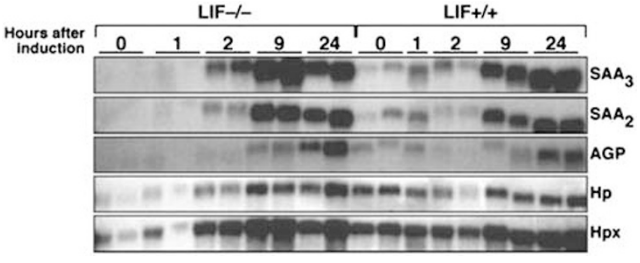

Figure 7 Acute-phase response transcripts in the liver of LIF−/− and mice upon LPS injection (10 μg, 0–24 h). RNA was extracted and hybridized with probes for SAA3, SAA2, AGP, Hp and Hpx as described under Materials and methods. While the basal level of acute-phase transcripts was significantly lower in LIF−/− mice, there was no difference at the transcriptional level upon LPS injection. The densitometric analysis of the gel is given in Table 2 ($P<0.05$). Mean values are given ($n=5$).

Table 4 Quantification of acute phase protein mRNA levels upon LPS injection

Time (h)	*HP*		*AGP*		*SAA2*		*SAA3*	
	LIF+/+	*LIF−/−*	*LIF+/+*	*LIF−/−*	*LIF+/+*	*LIF−/−*	*LIF+/+*	*LIF−/−*
0	49.6	**11.9**	28.3	**5.5**	175	**6.2**	159	**2.3**
2	35.7	26.3	12.4	11.5	189	194	228	349
9	59.7	70.1	34.1	47.7	727	708	841	1896
24	53.0	49.3	76.9	66.8	998	910	1182	774

Densitometric analysis of hepatic transcripts as arbitrary units, mean values ($n=2$). The values prior the LPS injections (in bold) are distinctly lower, while SAA3 levels at 2 and 9 h post-injection are increased in the LIF−/− mice.

IL-10 production and diminished basal expression of APP.

These results contradict previously published results,[15] where passive immunization with a polyclonal anti-LIF antiserum protected mice from LPS-induced shock. However, the functional properties of this rabbit IgG were not characterized. It is quite possible that the antiserum used increased the bioavailability of LIF rather causing its neutralization.[15] This interpretation would be coherent with our results, which are consistent with the data showing that an injection of LIF prior to LPS protected the mice from endotoxic shock.[11] Furthermore, we were able to attenuate LPS toxicity in LIF−/− mice by exogenous LIF.

The present results support the notion that endogenous LIF released during septic shock acts as an early mediator to dampen the inflammatory response.[14,28] The sustained systemic presence of TNFα in LIF−/− mice suggests that LIF could also have a regulatory effect on TNFα either at the level of transcription, translation or clearance. It has been shown that TNFα can substitute for endotoxin in inducing lethal endotoxemia in hypersensitivity models.[29,30] Inhibition of TNFα synthesis or release,[31] action and/or binding either through passive immunization[32] or interruption of its signalling pathway, as in the TNFRp55-deficient mice,[33] improves survival rates to endotoxic shock. In a cutaneous model of inflammation, LIF had an anti-inflammatory effect and it was shown to have regulatory roles on several cytokines including IL-1β and IL-6.[14] The observed prolonged and elevated serum concentration of IL-6 is most probably not a direct consequence of the LIF-deficiency, and may be related to increased TNFα activating IL-6 expression through the NF-κB signal transduction pathway.[33] Interestingly, a defective hypothalamic–pituitary–adreno-cortical system in response to inflammatory stress with elevated IL-6 and TNFα levels has been reported in LIF−/− mice.[3,28] This defect has been associated with reduced ACTH and corticosterone levels, and invites the hypothesis that LIF deficiency might be corrected in part by steroid replacement. Lastly, we asked whether the cytokine regulator IL-10 is regulated in a LIF-dependent manner. We show here that LPS-induced IL-10 synthesis is reduced in LIF−/− mice as compared to wild-type mice. The decreased IL-10 response *in vitro* and *in vivo* to LPS in LIF−/− mice is associated with increased TNFα levels. These results are consistent with studies showing a protective effect of IL-10 on endotoxic shock and TNFα expression.[27] Therefore, the data suggest that endogenous LIF favours the expression of IL-10 and thereby restricts TNFα production. In the absence of LIF, the synthesis of IL-10 is reduced allowing a largely unrestricted TNFα production and endotoxic shock.

APP have been suggested to be protective in endotoxic shock.[18,34] LIF−/− mice are capable to produce APP, although the response is altered. The mRNA transcript levels of APP genes are at lower basal levels in LIF−/− mice and the basal serum protein levels are slightly reduced. However, both transcripts and proteins were inducible and able to respond the same or even higher levels. The sustained protein expression SAP and to a lesser extent of SAA is probably due to the elevated IL-6 levels, which is a major inducer of APP synthesis. These results suggest that LIF plays a role in the establishment of normal base levels and in the induction of the early APP. As mortality in this endotoxic shock model usually appears within the first 10 h, the increased response of the APP occurs too late to have a mitigating effect.

Previously it has been shown that a single i.v. dose of LIF is able to protect mice from the lethal effect of LPS[12] and live *E. coli*.[11] The protection by prior administration of LIF does not necessarily have to be the same mechanism as the observed sensitization in the absence of LIF. It has been suggested that induction of the APP plays a central role in establishing protection. Similar results were achieved in experiments with prior injection of TNFα,[35] IL-1α,[36] IL-1β,[37] IL-6[38–40] and IL-10.[27] In all these studies, a decrease in TNFα production was observed, together with the ability to induce the synthesis of APP in the liver as having an important role of the involvement of the acute-phase response in protecting against lethal endotoxemia.

Interestingly, in IL-6-deficient mice, a similar increase in TNFα induction was reported as in LIF−/− mice.[41] It seems that LIF and IL-6 are able to compensate for each other's absence and induce a

similar set of APP. This shows again how closely related the shared biological activities of these two cytokines are, which may be due to the fact that both use the same signal transducing subunit gp130 receptor, which converts the low-affinity gp130 receptor to a high-affinity receptor by heterodimerization to a separate receptor subunits. The possible explanation suggested for the response in IL-6-deficient mice is that the absence of the negative IL-6 feedback mechanism is responsible for the heightened TNFα level, because IL-6 is able to inhibit TNFα transcription,[39,40] but does not explain the findings in our model, as IL-6 serum concentrations are also elevated. This is perhaps a further indication that the observed sensitivity is due to the lower concentrations of APP during the onset of endotoxemia. In addition, different individual APPs have shown to be protective in endotoxin-induced shock. These have included the third component of complement (C3),[42] α1-acid glycoprotein (possibly through its platelet aggregation inhibitory activity),[34] and α1-antitrypsin with its inhibition of serine proteinases preventing the proteolytic cleavage of the membrane-bound form of TNFα upon stimulation with LPS.[43]

In conclusion, our results show that LIF induction is necessary for a normal host reaction against endotoxin and probably other pathogen-derived molecules released in sepsis. Endogenous LIF mitigates the early endotoxic shock response by enhancing IL-10 and inhibiting TNFα and IL-6 synthesis and release, and inducing an acute-phase response.

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