Interferon γ Receptor Deficient Mice Are Resistant to Endotoxic Shock

By Bruce D. Car,* Vicki M. Eng,* Bruno Schnyder,*
Laurence Ozmen,‡ Sui Huang,§ Philipe Gallay,∥ Didier Heumann,∥
Michel Aguet,§ and Bernhard Ryffel*

*From the *Institute of Toxicology of the Swiss Federal Institute of Technology and University of
Zürich, 8063 Schwerzenbach; ‡Pharmaceutical Research, Hoffmann-La Roche, 4002 Basel;
§Molecular Biology, University of Zürich, Hönggerberg, 8093 Zürich; and the ∥Department of
Medicine, Division of Infectious Diseases, CHUV-BH19, CH-1101 Lausanne, Switzerland*

Summary

Antibody neutralization studies have established interferon γ (IFN-γ) as a critical mediator of endotoxic shock. The advent of IFN-γ receptor negative (IFNγR−/−) mutant mice has enabled a more direct assessment of the role of IFN-γ in endotoxin (lipopolysaccharide [LPS]-induced shock. We report that IFNγR−/− mice have an increased resistance to LPS-induced toxicity, this resistance manifesting well before the synthesis and release of LPS-induced IFN-γ. LPS-induced lymphopenia, thrombocytopenia, and weight loss seen in wild-type mice were attenuated in IFNγR−/− mice. IFNγR−/− mice tolerated 100–1,000 times more LPS than the minimum lethal dose for wild-type mice in a D-galactosamine (D-GalN)/LPS model. Serum tumor necrosis factor (TNF) levels were 10-fold reduced in mutant mice given LPS or LPS/D-GalN. Bone marrow and splenic macrophages from IFNγR−/− mice had a four- to sixfold decreased LPS-binding capacity which correlated with similar reduction in CD14. Serum from mutant mice reduced macrophage LPS binding by a further 50%, although LPS binding protein was only 10% reduced. The expression of TNF receptor I (p55) and II (p75) was identical between wild-type and mutant mice. Thus, depressed TNF synthesis, diminished expression of CD14, and low plasma LPS-binding capacity, in addition to blocked IFN-γ signaling in the mutant mice, likely to combine to manifest in the resistant phenotype of IFNγR−/− mice to endotoxin.

The gram-negative bacterial wall constituent, endotoxin (LPS), is the major active agent in the pathogenesis of septic shock (1). A shocklike state can be induced by a single injection of LPS into animals. This toxic syndrome, initiated after the entrance of LPS into the circulation, is mediated by macrophage-derived inflammatory cytokines. TNF-α appears to play a central role in the pathogenesis, as indicated by the inhibition of LPS-induced toxicity, by neutralizing anti-TNF-α antibodies (2, 3) by and the deletion of the TNF-type I receptor (4, 5).

IFN-γ exerts antiviral and immunostimulatory effects through macrophage and NK cell stimulation, and upregulates the expression of MHC class II antigens. IFN-γ is produced by activated T lymphocytes and NK cells and exerts its biologic activity through binding a unique cell surface receptor (6, 7). In previous investigations, it was shown that the administration of IFN-γ or neutralizing antibodies to IFN-γ modified the lethal outcome in several forms of endotoxic shock and gram-negative bacterial infections (8–17), clearly implicating its importance at the time of its synthesis in the pathogenesis of endotoxic shock.

The recent generation of IFNγR-deficient mice (18, 19) has allowed improved definition of the in vivo influence of IFN-γ on TNF production and endotoxic shock. We report that the absence of functional IFN-γ signaling in IFNγR−/− mice markedly reduces LPS-induced toxicity. Key observations contributing to this LPS resistance were lowered serum TNF levels and diminished expression of LPS receptors on macrophages/monocytes.

Materials and Methods

Animals. 7–10-wk-old 129 SV wild-type and IFNγR−/− mice bred in our animal facility (Institute of Toxicology of the Swiss Federal Institute of Technology) were used. Experimental groups consisted of 5–10 mice. The generation of these mice was recently described (18).

Reagents. LPS from *Escherichia coli* (serotype O111:B4) and FITC-conjugated LPS (serotype 0111:B4) were purchased from Sigma Chemical Co. (St. Louis, MO) and resuspended in pyrogen-free sterile saline. Rat anti–mouse monocyte-macrophage IgG (F4/80) was obtained from the American Type Culture Collection (Rockville, MD). Rat anti–mouse MAC-1 was purchased from

BMA Biomedicals (Augst, Switzerland). Goat anti–rat IgG conjugated to PE was from Southern Biotechnology Associates (Birmingham, AL). Rabbit anti–mouse TNFR I (p55) and II (p75) was the generous gift of Genentech (South San Francisco, CA). Rabbit anti–murine CD14 antibody was produced in the laboratory of Dr. Didier Heumann. D-galactosamine hydrochloride (D-GalN)[1] (Carl Roth GmbH & Co., Karlsruhe, Germany) was dissolved in saline immediately before use.

Determination of Serum TNF and LPS Binding Protein. Blood samples were obtained by retroorbital venipuncture. Serum concentrations of TNF were estimated by a cytotoxicity assay with WEHI-164 clone 13 cells as previously described (20). Results were expressed in nanograms per milliliter in reference to the cytotoxic activity of standard murine TNF-α. LPS binding protein (LPB) was determined by RIA as described (21). Standard murine TNF-α was obtained from Dr. W. Lesslauer (Hoffmann-La Roche AG, Basel, Switzerland).

Experimental Protocol. Mice were injected intraperitoneally with either LPS alone (1, 10, 30, 100, 500, and 1,000 μg/mouse) or LPS (0.1, 1, or 10 μg) in combination with D-GalN (20 mg) in a saline solution of 200 μl per dose. Blood was collected into heparinized tubes on the day before LPS administration for baseline values and at 1, 6, and 24 h after LPS challenge from animals anesthetized with methoxyflurane (Metofane; Pitman-Moore, Mundelein, IL). Blood plasma was separated immediately by centrifugation at 1,000 g for 10 min and was frozen at −20°C for batch processing. Preliminary experiments showed TNF to peak at 1 h when compared with 30 min, 2 h, and 4 h. Thereafter, all TNF measurements were performed on control and 1-h plasma. Body weight, clinical signs, and mortality were recorded at regular intervals.

Hematology and Clinical Chemistry. Heparinized blood was diluted (Cell Sheath SE-90L; Digitana, Switzerland) immediately after bleeding to minimize platelet aggregation, and standard hemograms were performed on a hematology analyzer (Sysmex E-2500; Digitana, Switzerland). Blood smears stained with Diff-Quik® (Dade, Düdingen, Switzerland) were read in parallel. Plasma aminotransferases were measured on a Cobas Fara Chemistry Analyzer (Hoffmann-La Roche) using kits from Boehringer Mannheim (Mannheim, Germany).

Flow Cytometric Analysis. Bone marrow cells from five wild type and five IFNγR−/− mice were obtained by flushing the femoral marrow into PBS/0.5% heparin, pelleting at 300 g, and washing twice in PBS/1% BSA (PBS) at 4°C. Spleen cells were isolated by passage through a size 80 mesh screen (Bellco Biotechnology, Vineland, NJ) and washing twice in PBS at 300 g. Rat anti–mouse F4-80 and CD11b IgG, and rabbit anti–mouse CD14 IgG were applied for 45 min, washed three times in PBS, and detected with goat anti–rat and goat anti–rabbit PE- and FITC-conjugated Ig (30 min), respectively, followed by two washes in PBS and resuspension for fluorescence analysis. LPS-FITC (1 and 10 μg/ml) was incubated with spleen and bone marrow cells in PBS in the presence of 10% pooled (eight animals) wild-type plasma, pooled IFNγR−/− murine plasma, or saline for 1 h at 4°C, followed by three washes in PBS and resuspension immediately before measurement. CD14 dependence of LPS-FITC binding was establishing using rabbit antiserum neutralizing to CD14. This antisera was able to completely prevent LPS-FITC binding as assessed by FACS® analysis. Immunofluorescence analysis was performed on a FACScan®

(Becton Dickinson & Co., Mountain View, CA) using LYSIS II software. Clear LPS-FITC binding of cells was restricted to F4-80, MAC-1 gated cells (macrophages).

Histology. Liver, kidneys, spleen, and lung were fixed in 4% buffered formaldehyde, cut at 5 μm, stained with hematoxylin and eosin, and evaluated microscopically.

Statistics. TNF levels, relative fluorescence, hematology values, and weights were compared using the nonparametric Wilcoxon's signed ranks tests. Mortality data were interpreted with the one-sided Fisher's exact test. P values <0.05 were considered statistically significant. Data are presented as mean ± standard error.

Results and Discussion

Abundant evidence for the pathogenic roles of TNF and IFN-γ in endotoxic shock and gram-negative infections has been obtained primarily through demonstration of protective effects after antibody neutralization of these cytokines (2–5, 8–17). The advent of mice deficient in their response to single cytokines has enabled the closer examination of potential toxic interactions between these cytokines. This latter approach has unequivocally identified TNF as a central mediator of endotoxic shock (4, 5). We present evidence confirming that IFN-γ is a key regulator of LPS toxicity. Although IFN-γ protein and mRNA peak levels occur only 4–6 h after LPS administration (13, 22, 23) we demonstrate mitigated toxicity already at 1 h after injection (reduced weight loss and thrombocytopenia) with marked differences at 6 h, suggesting the presence of important IFN-γ-mediated or primed signaling events early in the development of the toxic state. We therefore examined parameters likely to be of early pathogenic importance in order to elucidate the marked resistance of the IFNγR−/− mice to endotoxin.

Resistance to Endotoxic Shock of IFNγR−/− Mice. Wild-type mice receiving only 10 μg LPS appeared distressed and had watery diarrhea within 6 h of LPS injection, whereas IFNγR−/− mice tolerated up to 100 μg without demonstrating clinical changes. At LPS doses >500 μg dose-dependent differences are lost between IFNγR−/− and IFNγR+/+ mice (data not shown), as is also the case for TNFRI (P55) deficient mice and their wild-type counterparts (4). Correlating with clinical appearance was a weight
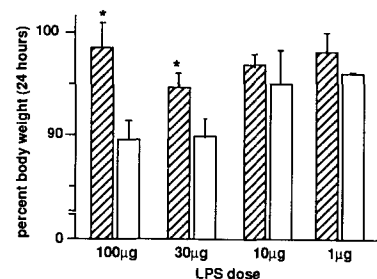
Figure 1. Body weight loss after LPS injection. LPS (1–100 μg/mouse) was injected intraperitoneally into wild type (*open bar*) or IFNγR−/− mice (*crosshatched bars*). Body weights were recorded before and 24 h after injection. Data are presented as mean percent of control body weight (100%) over 24 h. Three to five mice were used per group, mean ± SE given. (*$p < 0.02$).
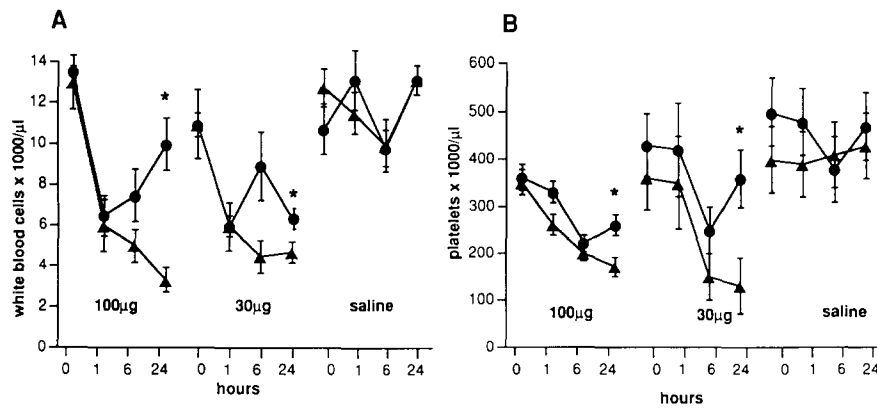
Figure 2. Hematological alterations after LPS administration. Leukocyte (A) and thrombocyte (B) counts at 1, 6, and 24 h after LPS injection (0, 30, and 100 µg/mouse). (A) Total white blood cells (1,000/µl) in wild-type (▲) and IFNγR−/− mice (●) after LPS or saline injection. All treated mice became leukopenic. For differential leukocyte evaluation see Table 1. Partial recovery by IFNγR−/− mice can be seen at 24 h (*p <0.02). Mean ± SE given. (B) Platelet counts (1,000/µl) in wild-type (▲) and IFNγR−/− mice (●) after LPS and saline injection. Mild thrombocytopenia is already evident at 1 h. Recovery of platelet counts occurred in LPS-treated mutant mice by 24 h (*p <0.05). Mean ± SE given.

loss, already apparent at 1 h in the 100-µg wild-type group (data not shown), which was marked at 24 h (Fig. 1). A severe weight loss (10% of body weight) was registered at 24 h in wild-type mice receiving the two highest LPS doses (p <0.02 wild-type versus mutant mice). Body weight loss was referable to severe dehydration due to diarrhea.

Mice from all groups treated with LPS became leukopenic to a similar degree of severity within 1 h of LPS injection (Fig. 2 A). The leukopenia observed at 6 h comprised a severe absolute lymphopenia, monocytopenia, disappearance of eosinophils from peripheral blood, and an absolute neutrophilia (Table 1). This leukocyte pattern was independent of dose (data not shown). Neutrophila was more pronounced and leukocytosis less severe in negative mice (p <0.05) (Table 1). Hematologic alterations were similar in mice treated with LPS alone or with LPS and D-GalN (data not shown). Marked toxic change with basophilic coloration, fine vaculation and Döhle bodies, and a left shift with stages to metamyelocytes was evident in neutrophils of mutant and normal mice. The recovery of total leukocyte counts at 24 h was significantly more rapid in IFNγR−/− mice than in wild-type mice (p <0.02) with a clear trend apparent at 6 h (Fig. 2 A).

Thrombocytopenia was already evident at 1 h, with levels dropping progressively for 24 h in wild-type mice (p <0.05), whereas most IFNγR−/− mice reached nadir at 6 h with mild recovery of platelet number being observed at 24 h (Fig. 2 B). Thus, in the absence of a functional IFNγR system, hematologic and clinical signs of LPS toxicity are mitigated. The higher neutrophil count in LPS-treated IFNγR−/− mice probably reflects reduced extravasation of neutrophils in response to reduced production of chemokines (IL-8–like peptides). TNF levels are reduced and IFN-γ is nonsignaling in IFNγR−/− mice; both are critical signals for the induction of chemokine synthesis and secretion in extravascular tissues (24).

IFNγR−/− Mice Are Resistant to Endotoxic Shock and Hepatocellular Necrosis in the D-GalN Model. Wild-type mice administered ⩾0.1 µg LPS in combination with D-GalN succumbed to acute liver failure 6–24 h after injection. In sharp contrast, 10 µg LPS with D-GalN was lethal for only 25% of IFNγR−/− mice (Table 2). Thus, 100–1,000 times more LPS was required to produce an equivalent outcome in IFNγR−/− mice. The degree of protection observed in the TNFR I-deficient mice with the LPS/D-GalN model (4, 5)

Table 1. *Differential Leukocyte Counts in LPS-treated IFNγR − / − and IFNγR + / + Mice*

Mouse	Group	WBC	PMN	M	L	E
IFNγR − / −	Untreated*	12.5 ± 2.0	0.7 ± 0.2	0.4 ± 0.1	11.1 ± 1.7	0.3 ± 0.1
IFNγR + / +	Untreated*	13.9 ± 1.3	0.8 ± 0.1	0.4 ± 0.2	12.4 ± 0.8	0.3 ± 0.1
IFNγR − / −	1 µg LPS‡	7.1 ± 0.4§	3.4 ± 0.1‖	0.02 ± 0.01	3.7 ± 0.4¶	0
IFNγR + / +	1 µg LPS‡	5.7 ± 0.6§	2.3 ± 0.3‖	0.03 ± 0.01	3.2 ± 0.4¶	0

Values are mean ± SE of the mean and represent 1,000 cells/µl blood.
* Untreated mice received 20 mg D-GalN in saline only.
‡ LPS administered with 20 mg D-GalN; blood taken at t = 6 h.
Wilcoxon's ranked sum test, comparison between + / + and − / − animals:
§ p <0.05
‖ p <0.05
¶ p = 0.13.
E, eosinophils; L, lymphocytes; M, monocytes; PMN, neutrophils; WBC, white blood cells.

Table 2. *Mortality in IFNγR −/− and IFNγR +/+ Mice after LPS/D-Gal Administration*

D-GalN	LPS	IFNγR −/− Dead/group	IFNγR +/+ Dead/group
mg	*μg/mouse*		
20	0	0/5	0/5
20	0.01	0/8	0/8
20	0.1	0/8*	7/8
20	1	0/8*	8/8
20	10	2/8*	8/8
0	10	0/4	0/4

Mice received indicated dosages of D-GalN, *E. coli* LPS (0111:B4) intraperitoneally in saline. All deaths indicated occurred within 12 h of injection. Surviving animals were observed for 1 wk. Experiment was repeated three times with consistent outcome (typical experiment given). * Fisher's exact test (one sided) p <0.02, demonstrating statistical difference in overall survival rate between IFNγR −/− and IFNγR +/+ mice groups.

is similar to that of the IFNγR−/− mice in this study. 100-fold enhancement of sensitivity to LPS injection achieved through chronic bacille Calmette-Guérin (BCG) infection was also recently used to demonstrated protection from lethality in IFNγR−/− mice (25). Alanine (ALT) and aspartate (AST) aminotransferases, enzyme markers of hepatocellular necrosis, were elevated in all wild-type mice after 6 h of LPS treatment, an effect significantly reduced in IFNγR−/− mice (Table 3). The elevation of ALT and AST seen in staphylococcal enterotoxin (SEB)–treated IFNγR+/+ mice was completely suppressed in IFNγR−/− mice (Table 3), suggesting that T cell activation–induced systemic toxicity is an event medi-

ated at least in part through the IFNγR, as well as partly through the TNFRI, since similar protection was also observed in SEB-treated TNFRI deficient mice (5). Markedly elevated aminotransferases in wild-type animals were associated with a destruction of hepatocytes, characterized by widespread pyknosis and karyorrhexis of hepatocyte nuclei, and cellular fragmentation (Fig. 3 *A*). Surviving IFNγR−/− mice demonstrated only mild microvacuolar centrilobular degeneration and some cell dropout (Fig. 3 *B*). Since liver failure was markedly attenuated in IFNγR−/− mice, IFNγR-receptor-dependent events are clearly important for the generation of the acute liver necrosis in LPS/D-GalN toxicity.

LPS-induced Serum TNF Levels in IFNγR−/− Mice Are Reduced. LPS administered together with D-GalN induced a significant, dose-dependent synthesis and release of TNF in all animals, which was 10-fold less in IFNγR−/− mice (Fig. 4 *A*). This clear difference was restricted to doses of LPS ≤30 μg per mouse. Lack of clear dose-responsive toxicity at high LPS levels was also observed in TNFRI mice (4). Similar TNF levels were obtained in mice injected with LPS alone (Fig. 4 *B*). Since serum TNF was not detectable 4 h after injection in either wild-type or IFNγR−/− mice, delayed synthesis can be excluded. After chronic BCG infection and subsequent LPS sensitization, LPS treatment (25 μg per mouse) was recently shown to result in synthesis of TNF-α and IL-1α, 100- and 12-fold lower in IFNγR−/− mice, respectively (25). The observation that neutralization of IFN-γ in mice immediately before infection with *E. coli* reduced mortality without decreasing TNF levels, but that administering of IFN-γ enhanced both mortality and TNF, suggests that the mechanism of TNF inhibition in IFNγR−/− mice is perhaps different than that documented for antibody neutralization, and at least in models using live bacteria, IFN-γ alone rather than TNF and IFN-γ is critical in determining

Table 3. *Transaminase Serum Levels: LPS and SEB-treated IFNγR −/− and IFNγ +/+ Mice*

| D-GalN	Group		AST		ALT	
			IFNγR −/−	IFNγR +/+	IFNγR −/−	IFNγR +/+
mg/kg	*μg/mouse*					
20	0		143 ± 9	131 ± 37	78 ± 13	68 ± 15
20	0.01	LPS	144 ± 17	118 ± 16	50 ± 6	46 ± 29
20	0.1	LPS	209 ± 50	280 ± 75*	96 ± 32	222 ± 167‡
20	1	LPS	217 ± 33	863 ± 288‡	68 ± 18	1,817 ± 402‡
20	10	LPS	153 ± 110	593 ± 197‡	282 ± 78	2,040 ± 309‡
20	100	SEB	149 ± 8	316 ± 46§	60 ± 16	143 ± 23§
0	100	SEB	68 ± 12	166 ± 20	39 ± 7	39 ± 21

All AST ALT results represent mean values ± SE from five to eight animals. Experiment was repeated three times with consistent outcome (typical experiment given, same as Table 2). Normal range (±2 SD) AST = 54–170 U/liter, Normal range ALT = 32–114 U/liter.
Wilcoxon's signed ranks test, comparison between IFNγR −/− and IFNγR +/+ mice of each group:
* p <0.01.
‡ p <0.04.
§ p = 0.09.
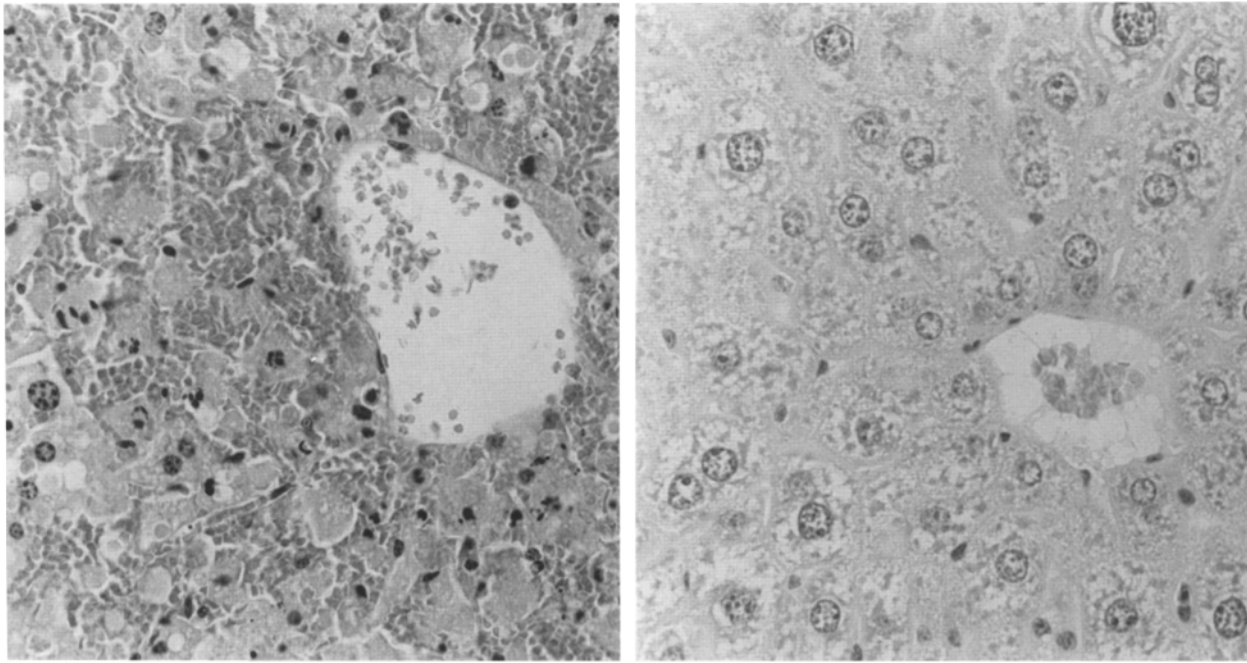
Figure 3. Liver necrosis in the LPS/D-GalN model. (A) Necrosis, pyknosis, and karyorrhexis of hepatocytes in wild-type mouse given 1 μg LPS and 20 mg/kg D-GalN. Death observed at 7 h. (6 h AST, 1,435 U/liter; ALT, 3,233 U/liter.) (B) IFNγR−/− mouse given same dose with normal morphology (euthanized at 7 h, 6 h AST, 119 U/liter; ALT 113 U/liter) (formalin fixed, hematoxylin-eosin stained). ×200.

mortality (16, 17). The deficiency status of the IFNγR−/− mice would interrupt any homeostatic mechanisms dependent on IFN-γ more thoroughly than short-term antibody neutralization, and likely resulted in the loss of priming mechanisms necessary for the normal production of TNF in this study. Previous investigations have shown that LPS-stimulated macrophages produce increased amounts of TNF when treated concomitantly with IFN-γ, which is regulated at the the level of TNF gene transcription and possibly of mRNA stability (26–29). IFN-γ reportedly enhances the expression of TNFR

on several cell types by three- to fivefold (30–32). Since TNF induces its own synthesis in macrophages (33), low expression of TNFR could have potentially contributed to deficient TNF synthesis in IFNγR−/− mice, however flow cytometric analyses of TNFRI and II (Table 4) showed a remarkably consistent level of receptor expression between mutant and wild-type mice. The possibility that reduced TNF production in IFNγR−/− mice was referable to decreased numbers of fixed macrophages was addressed by performing immunohistochem-
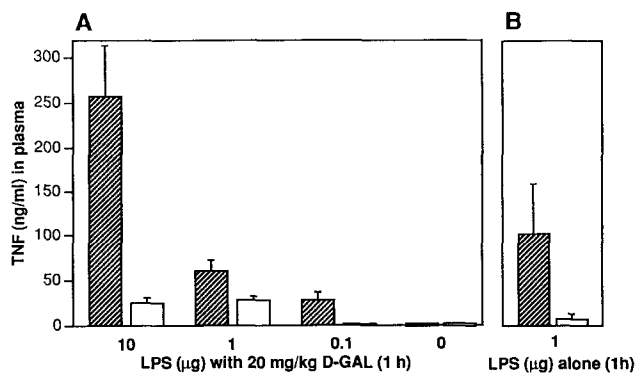
Figure 4. Serum TNF concentration 1 h after LPS and D-GalN (A) or LPS (B) injection. Wild-type mice (*crosshatched bars*) demonstrating markedly higher TNF levels in LPS/D-GalN model (A) and LPS alone (B) than IFNγR−/− mice (*open bars*). D-GalN (alone)-treated mice produced no TNF. Mean ± SE.

Table 4. *Expression of TNFR and CD14 in Monocytes/ Macrophages* of IFNγR +/+ and IFNγR −/− Mice*

Receptor	Cell source	IFNγR +/+ Mean FU	IFNγR −/− Mean FU
TNFRI	Bone marrow	13.2 ± 1.2‡	12.2 ± 1.0
TNFRII	Bone marrow	8.5 ± 0.6	7.5 ± 0.9
CD14	PBMC	12.4§	4.3
CD14	Peritoneum	71.5	11.6
CD14	Bone marrow	74.1	9.3

* Monocytes/macrophages gated for with F4-80, and CD11b.
‡ Data expressed in mean fluorescence units (five mice per group) with background fluorescence subtracted. Standard error is given.
§ Where means are not given data represent a typical result from a series of at least three consistent independent experiments.
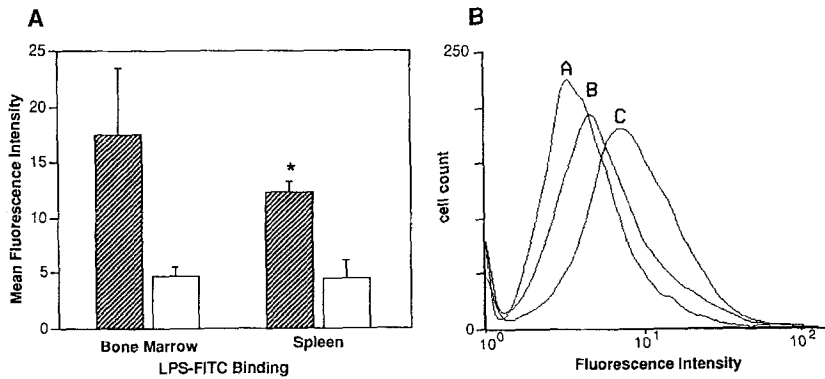
Figure 5. LPS receptor expression on macrophages. (*A*) Mean fluorescence (LPS-FITC binding) of bone marrow and splenic macrophages of wild-type mice (*crosshatched bars*) is markedly higher than that of IFNγR−/− mice (*open bars*). n = five mice, *p <0.05. LPS-FITC binding was only detectable in F-480 positive macrophages. Binding was carried out in the presence of 10% wild-type plasma in PBS/1% BSA for 60 min (see Materials and Methods). (*B*) Mean fluorescence intensity of macrophages (wild-type cells) demonstrating >50% reduction in LPS-FITC binding in the presence of 10% pooled (IFNγR−/−) plasma (*B*), compared with 10% pooled IFNγR+/+ plasma (*C*). Cells incubated without plasma (*A*) demonstrate marked reduction in fluorescence intensity. Binding could be completely inhibited with antisera to mouse CD 14 (data not shown).

ical staining for F4-80 antigen-positive cells in spleen and liver, which demonstrated identical distribution, morphology, and numbers of macrophages in mutant and wild-type mice (data not shown). The dependence of LPS-induced effector functions of macrophages on IFN-γ, which thus contributes to the toxicity of LPS, has been hypothesized (33). The dramatic reduction of LPS-induced synthesis and release of TNF into the serum of IFNγR−/− mice confirms the hypothesized role of IFN-γ for TNF synthesis.

Impaired Macrophage Recognition of LPS by IFNγR−/− Mice. Since TNF levels were higher in wild-type mice, and given that TNF appears much earlier (1 h) than IFN-γ (4–6 h) in an endotoxic shock response (13, 22, 23), it appeared likely that monocyte-macrophages of wild-type mice were more sensitive to LPS than their mutant counterparts, particularly since equivalent numbers of monocytes are present in wild-type and IFNγR−/− mice (18). We examined the binding of LPS-FITC to spleen and bone marrow macrophages in the presence and absence of plasma pooled from untreated wild-type mice, in light of the recent reports that LPB enhances the binding of LPS to the murine CD14 receptor (34, 35). Wild-type macrophages possessed a four- to sixfold higher binding capacity for LPS-FITC than macrophages from IFNγR−/− mice (Fig. 5 *A*, p <0.05). Consistent with this result was a four-to sevenfold higher CD14 expression (Table 4). Macrophages demonstrated LPS-FITC binding that was markedly enhanced by the presence of plasma (Fig. 5 *B*), and inhibitable by anti-CD14 antisera (data not shown). These results suggest an apparent in vivo upregulation of the CD14 receptor by the low levels of IFN-γ presumably present nor-

mally in mice, which contrasts in vitro with data showing that human IFN-γ is able to markedly downregulate the expression of human monocyte CD14, particularly in the presence of LPS (36, 37). This downregulation is likely a high dose-dependent effect that, after the systemic release of IFN-γ in the presence of LPS, contributes to the turning off of LPS-mediated events.

Plasma-mediated enhancement of LPS-FITC binding was more than 50% reduced in macrophages preincubated with pooled plasma from IFNγR−/− mice (Fig. 5 *B*), suggesting that LPB is also reduced in these mice. RIA for LPB from a serum pool of eight positive mice, however, yielded 2.2 ± 0.2 μg/ml, and that of negative mice, 2.0 ± 0.3 μg/ml, which does not explain the observed reduction of LPS-FITC binding. This may suggest the presence of additional factors in murine plasma capable of promoting LPS-binding to macrophages. LPS-FITC binding was restricted to F4-80 positive macrophages, which also expressed MAC-1 (CD11b). Downregulation of CD14, LPB function, and the mechanism of reduced TNF synthesis in IFNγR−/− mice are presently under investigation.

In conclusion, we report that the toxicity of LPS is significantly reduced in IFNγR−/− mice, which are able to withstand the deleterious effects of 100–1,000 times more LPS in the D-GalN sensitization model than wild-type mice. The combination of defects present in IFNγR−/− mice, including reduced TNF synthesis, impaired LPS recognition due to diminished CD14 expression and plasma-facilitated receptor binding, and blocked IFN-γ signaling, act in concert to seriously impair LPS-induced toxicity.

Address correspondence to Dr. B. Ryffel, Institute of Toxicology, ETH and University of Zürich, Schorenstr. 16, 8603 Schwerzenbach, Switzerland.

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