Genetic Deletion of the Tumor Necrosis Factor Receptor p60 or p80 Sensitizes Macrophages to Lipopolysaccharide-induced Nuclear Factor-κB, Mitogen-activated Protein Kinases, and Apoptosis

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LPS mediates a number of biologic manifestations of sepsis, including fever, hypotension, multiple organ failure, shock, and death (3). These effects of endotoxin are believed to result from an uncontrolled production of proinflammatory cytokines produced by cells of the reticuloendothelial system, particularly macrophages. LPS-dependent macrophage activation results in the release of TNF, IL-1, IL-6, IL-8, IL-10, and IL-12.

LPS interacts with most cells through CD14, a 55-kDa glycosylphosphatidylinositol-anchored protein expressed on the surface of monocytes and neutrophils (4, 5). The binding of LPS to CD14 is enhanced by the LPS-binding protein present in the serum (5, 6). Mice that lack the CD14 gene show resistance to LPS-induced shock (7). LPS is then transferred to the transmembrane signaling receptor toll-like receptor 4 (TLR4) and its accessory protein MD2 (8–10). LPS stimulation of human monocytes activates several intracellular signaling pathways that include the IκB kinase (IKK)-NF-κB pathway (11–15) and three mitogen-activated protein kinase (MAPK) pathways: p42/p44ERKMAPK extracellular signal-regulated kinases 1 and 2 (36–38), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) (22), and p38MAPK (23, 24).

Both LPS and TNF display several overlapping and nonoverlapping cellular responses. TNF induces apoptosis in a wide variety of tumor cells (for references see Ref. 25), whereas LPS is known to induce apoptosis only in certain types of endothelial cells (26). Like TNF, however, LPS also stimulates ceramide release (27), activates ceramide-activated protein kinase (28), and caspase-1 (29), and induces the SAPK/JNK pathway (17). Both LPS and TNF activate the nuclear transcription factor NF-κB but through pathways consisting of similar and dissimilar steps (30). For instance the inhibitory subunit of NF-κB, IκBα, is more profoundly affected by LPS than by TNF, whereas IκBβ is affected equally by both agents (31).

Although LPS is a potent inducer of TNF and some of the apoptotic effects of LPS are mediated through TNF (32), we have shown that through the activation of NF-κB, LPS can suppress TNF-induced apoptosis (33). How TNF modulates LPS-induced cell signaling is not known. Genetic deletion of TNF receptor 1 (also called p60) or TNF receptor 2 (also called p80) has been shown to protect mice from low doses of LPS but not from high doses (34–36). Whether genetic deletion of TNF receptor 1 or 2 also affects the LPS-mediated signaling is not known. In this report, we used macrophages derived from wild type (wt) mice and from mice with genetic deletions of the type 1 receptor gene (p60/−), the type 2 receptor gene (p80/−), or both receptor genes (p60/− p80/−) (37). Our goal was to investigate the effect of these genetic deletions on LPS-mediated activation of NF-κB, MAPKs, and apoptosis. Our results show that the deletion of TNF receptors sensitizes the cells to LPS-induced signaling, thus providing evidence of cross-talk.
EXPERIMENTAL PROCEDURES

Materials—LPS (Escherichia coli, 055: B5) and 3-(4,5-dimethyl-2-yl)-2,5-diphenyl-[2H]-tetrazolium bromide (MTT) were purchased from Sigma. Penicillin, streptomycin, RPMI 1640, fetal bovine serum were obtained from Invitrogen (Carlsbad, CA). Antibodies to the bovine serum, 100 units/ml penicillin, and 100 units/ml streptomycin were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum were obtained from Invitrogen (Carlsbad, CA). Antibodies against the phospho-p38MAPK, phospho-ERK1/2, and ERK1/2 were obtained from Cell Signaling Technology (Beverly, MA). Antibody against CD14 was purchased from Cell Sciences (Norwood, MA).

Cell Lines and Culture—Mice with genetic deletion of p60, p80, or both TNF receptors have been described (34, 35). p80−/− and p60−/− p80−/− mice were obtained from Genentech Inc. (San Francisco, CA), and p60−/− mice were obtained from Jackson Laboratories (Bar Harbor, ME). Immortalized macrophage cell lines were established from the bone marrow of wt C57BL/6J mice and its TNF receptor homozygous mice (p60−/−, p80−/−, and p60−/− p80−/−) as previously described (37). By using reverse transcription-PCR, fluorescence-activated cell sorter analysis, and Western blot analysis, the cells have been shown to lack expression of TNF receptors as expected (37). All cell lines described (37). By using reverse transcription-PCR, fluorescence-activated cell sorter analysis, and Western blot analysis, the cells have been shown to lack expression of TNF receptors as expected (37). By using reverse transcription-PCR, fluorescence-activated cell sorter analysis, and Western blot analysis, the cells have been shown to lack expression of TNF receptors as expected (37). By using reverse transcription-PCR, fluorescence-activated cell sorter analysis, and Western blot analysis, the cells have been shown to lack expression of TNF receptors as expected (37).

Cytotoxicity Assay (MTT Assay)—The cytotoxic effects of LPS were determined by the MTT uptake method as described (38). This assay utilizes the tetrazolium dye, MTT, which is converted enzymatically in mitochondria of viable cells to a blue dye that is insoluble in water. The resulting crystalline formazan deposits are then solubilized in the extraction buffer (20% SDS in 50% N,N-dimethylformamide), and absorbance is measured at 570 nm.

Briefly, 5000 cells were incubated in duplicate in 96-well plates in the presence of LPS for 72 h at 37 °C. Thereafter, the MTT solution was added to each well. After a 2-h incubation at 37 °C, extraction buffer was added, the cells were incubated overnight at 37 °C, and then the optical density was measured at 570 nm using a 96-well multiscan (Dynex Technologies, MRX Revelation, Chantilly, VA).

Western Blot Analysis—30–50 μg of cytoplasmic protein extract, prepared as described (39), was resolved on SDS-PAGE. Then the proteins were electrotransferred to a nitrocellulose membrane, blocked with 5% nonfat dry milk, and probed with first antibodies for 2 h at 4 °C. The blotting membrane was washed and exposed to horseradish peroxidase-conjugated secondary antibodies for 1 h, and the blots were finally detected by ECL (Amersham Biosciences). For first antibody, we used anti-phospho-p38MAPK, phospho-ERK1/2, p38MAPK, ERK1/2, iNOS, COX2, CD14, TLR4, and β-actin antibodies.

Electrophoretic Mobility Shift Assay (EMSA)—NF-κB activation was assessed by EMSA as described previously (40). In brief, nuclear extracts prepared from LPS-treated or untreated cells were incubated with 32P endabeled 45-mer double-stranded NF-κB oligonucleotide from human immunodeficiency virus-1 long terminal repeat (5’-TTGTT-TACAAGGAGACTTCCGTTGAGATCTCTCCGAGGGCGGTTG-3’; the underlined sequence is the binding site) for 30 min at 37 °C, and the DNA-protein complex was resolved in a 6.6% native polyacrylamide gel. The specificity of binding was examined by competition with unlabeled 100-fold excess oligonucleotide and with mutant oligonucleotide. The composition and specificity of binding was also determined by supershift of the DNA-protein complex using specific and irrelevant antibodies. For supershift experiment, the antibody-treated samples of NF-κB were resolved in a 5.0% native gel. The radioactive bands from the dried gels were visualized and quantitated by phosphorimaging (Molecular Dynamics, Sunnyvale, CA) using ImageQuant software.

IKK Assay—The IKK assay was performed by a method described previously (41). Briefly, IKK complex from cytoplasmic extract was precipitated with antibody against IKKα followed by treatment with protein A/G-Sepharose beads (Pierce). After a 2-h incubation, the beads were washed with lysis buffer and then assayed in kinase assay mixture containing 50 mM HEPES (pH 7.4), 20 mM MgCl2, 2 mM dithiothreitol, 20 μCi of (γ-32P)ATP, 10 μM unlabeled ATP, and 2 μg of substrate GST-IκBα. After incubation at 30 °C for 30 min, the reaction was terminated by boiling with SDS sample buffer for 5 min. Finally, the protein was resolved on 10% SDS-PAGE, the gel was dried, and the radioactive bands were visualized by phosphorimaging. To determine the total amounts of IKK in each sample, 30 μg of the cytoplasmic extract was resolved by 7.5% SDS-PAGE, electrotransferred to nitrocellulose membrane, and then blotted with either anti-IKKα or IKKβ antibodies.

c-Jun N-terminal Kinase Assay—The c-Jun N-terminal kinase assay was performed by a modified method as described earlier (38). Briefly, 200 μg of whole cell extract was treated with anti-IKKα antibody, and the immune complexes so formed were precipitated with protein A/G-Sepharose beads (Pierce). The kinase assay was performed using washed beads as source of enzyme and GST-Jun (1-79) as substrate (2 μg/sample) in the presence of 10 μCi of (γ-32P)ATP/sample. The kinase reaction was carried out by incubating the above mixture at 30 °C in the kinase assay buffer for 30 min. The reaction was stopped by adding SDS sample buffer, followed by boiling. Finally, protein was resolved on a 10% reducing gel. The radioactive bands of the dried gel were visualized and quantitated by phosphorimaging as described above.

RESULTS

Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced Activation of NF-κB—Activation of NF-κB is one of the earliest events induced by LPS in most cells. We investigated whether LPS-induced NF-κB activation is modulated by individual TNF receptors. We treated wt macrophage cell line and its TNF receptor-deficient variants with LPS, prepared the nuclear extracts, and analyzed them by EMSA for NF-κB.

Dose-dependent activation of NF-κB occurred in wt cells, in single-gene knockout cells, and in double-gene knockout cells (Fig. 1A, left panels). The level of NF-κB activation, however, varied. Maximum activation observed with wt, p60−/−, p80−/−, and p80−/− p60−/− cells was 3.2-, 8.7-, 7.1-, and 9.7-fold, respectively. As shown in Fig. 1A (right panels), time-dependent activation of NF-κB occurred in all cell types, but again the level of NF-κB activation varied. Maximum activation observed with wt, p60−/−, p80−/−, and in p80−/− p60−/− cells was 4.7-, 10.4-, 9.6-, and 9.9-fold, respectively. The dose response and time course of LPS-induced NF-κB activation clearly show that wt cells are least sensitive to LPS, and those with TNF receptor deletion (p60, p80 or both) are maximally sensitive (Fig. 1B).

EMSA of nuclear extracts prepared from LPS-treated cells showed that either anti-p50 or anti-p65 antibodies supershifted the NF-κB-DNA complex, whereas preimmune serum or irrelevant antibody was ineffective (Fig. 1C).

Due to the presence of a 100-fold excess of unlabeled κB-oligonucleotide (Fig. 1C, Competitor) but not by mutant oligonucleotide (Mutant oligo). Additionally, when compared with wt, the p60−/−, p80−/−, and p80−/− p60−/− cells showed an induction of an additional NF-κB band by LPS (Fig. 1A). This band consisted of p50-p50 homodimer as revealed by supershift analysis (data not shown).
Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced Activation of IκB—Activation of NF-κB requires the activation of IKK. We investigated whether LPS-induced IKK activation is modulated by individual TNF receptors. We treated wt and TNF receptor-deficient variants with 0.1 μg/ml of LPS for different times, prepared the whole extracts, and analyzed them for IKK by immune complex kinase assays. As shown in Fig. 2, time-dependent activation of NF-κB occurred in wt cells and in all TNFR knockout cells. The level of IKK activation, however, varied. Maximum activation observed with wt, p60−/−, p80−/−, and p60−/−p80−/− cells was 2.5-, 3.1-, 5.8-, and 4.5-fold, respectively. The kinetics of IKK activation was slightly slower in wt cells (15 min) than in TNF receptor-deleted cells (15 min). The lower panels represent loading controls and indicated that IKK activation in cells was not due to a change in the expression of IKKα and IKKβ proteins. These results suggest that LPS-induced IKK activation was enhanced by the deletion of the TNF receptors, and this enhancement correlated with NF-κB activation.

Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced NO Production—The LPS-iNOS in macrophages is known to be regulated by NF-κB (43). We first determined whether TNF receptor had any effect on LPS-induced NO production in macrophages. Macrophages were cultured for 48 h in the presence of different concentrations of LPS, and NO production was assayed by using Griess reagent. LPS induced NO production in a dose-dependent manner in all macrophage cell lines, but the induction was lowest in wt cells and highest in cells where both TNF receptors were deleted (Fig 3A). Maximum induction observed was 12.6-, 20.1-, 39.6-, and 45.5-fold in wt, p60−/−, p80−/−, and p60−/−p80−/− cells at an LPS concentration of 1 μg/ml.

We also investigated the effect of TNF receptors on LPS-induced iNOS protein expression. The cells were treated with 0.01 μg/ml of LPS for different times or with various concentrations of LPS for 24 h, and iNOS expression was determined by Western blot analysis. LPS induced iNOS expression in a dose- and time-dependent manner (Fig. 3B). The induction was less in wt and in p60−/− cells than in p80−/− or p60−/−p80−/− cells. These results are in agreement with those for NO production and demonstrate that TNF receptors suppressed LPS-induced activation of macrophages. The deletion of p80 receptor had a more pronounced effect on LPS-induced NO production and iNOS expression than deletion of the p60 receptor.

Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced COX2 Expression—COX2 is another inflammatory gene that is regulated by NF-κB and induced by LPS (44). We investigated whether LPS-induced COX2 expression is modulated by TNF receptors. Western blot analysis indicated that LPS induced COX2 expression in a dose- and time-dependent manner (Fig. 4). The induction was least in wt and p60−/− cells...
and greatest in \( \text{p80}^{-/-} \) and \( \text{p60}^{-/-}\text{p80}^{-/-} \) cells. These results demonstrate that the presence of TNF receptors suppressed LPS-induced activation of macrophages.

**Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced Activation of JNK**—Activation of JNK is one of the earliest events induced by LPS in most cells (22). To explore the specific role of TNF receptors in LPS-induced JNK activation, we treated the wt macrophage cell line and its TNF receptor-deficient variants with LPS (0.1 \( \mu \)g/ml) for various times, prepared whole cell extracts, immunoprecipitated the JNK, and analyzed them for JNK by immune complex kinase assay. Time-dependent activation of JNK occurred in all cell types (Fig. 5), but the level of JNK activation varied. Maximum activation observed with wt, \( \text{p60}^{-/-} \), \( \text{p80}^{-/-} \), and \( \text{p80}^{-/-}\text{p60}^{-/-} \) cells was 2-, 3.4-, and 4.2-fold, respectively. Thus, our results suggest that activation of a kinase by LPS is modulated by both TNF receptors.

**Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced Activation of \( \text{p38 MAPK} \)**—Like JNK, \( \text{p38 MAPK} \) is a Ser/Thr protein kinase activated rapidly by LPS (23). To explore the specific role of TNF receptors in LPS-induced \( \text{p38 MAPK} \) activation, we treated the wt macrophage cell line and its TNF receptor-deficient variants with LPS (0.1 \( \mu \)g/ml) for various times and performed Western blot analysis using phospho-(Tyr/Thr)-specific \( \text{p38 MAPK} \) antibodies. As shown in Fig. 6, time-dependent activation of \( \text{p38 MAPK} \) occurred in all cell types. Maximum activation observed with wt, \( \text{p60}^{-/-} \), \( \text{p80}^{-/-} \), and \( \text{p80}^{-/-}\text{p60}^{-/-} \) cells was 2.6-, 3.4-, and 4.2-fold, respectively. Once again, our results suggest that activation of a kinase by LPS is modulated by both TNF receptors.

**Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced Activation of \( \text{ERK1/2} \)**—Through the Ras/Raf/\( \text{MAPK} \) kinase cascade, LPS can activate \( \text{ERK1/2} \) (21). To explore the
specific role of TNF receptors in LPS-induced ERK1/2 activation, we treated the wt macrophage cell line and its TNF receptor-deficient variants with LPS (0.1 μg/ml) for different times and performed Western blot analysis using phospho-(Tyr/Thr)-specific ERK1/2 antibody. As shown in Fig. 7, time-dependent activation of ERK1/2 occurred in all cell types. Maximum activation observed with wt, p60−/−, p80−/−, and p80−/− p60−/− cells was 1.6-, 1.6-, 3.1-, and 3.1-fold, respectively. Activation could be seen as early as 5 min. Thus, activation of still another kinase by LPS is modulated by both TNF receptors.

Deletion of TNF Receptors Sensitizes Macrophages to LPS-induced Apoptosis—LPS is known to induce apoptosis in certain cell types (26, 32). To determine the effect of TNF receptors on LPS-induced cytotoxicity, all cell types were incubated for 72 h in the presence of different concentrations of LPS, and then cell viability was assayed by MTT uptake. LPS decreased cell viability in all cell types in a dose-dependent manner (Fig. 8A). The maximum cytotoxicity observed on treatment of wt, p60−/−, p80−/−, and p80−/− p60−/− cells with 1 μg/ml LPS was 10, 50, 50, and 70%, respectively. As little as 0.01 μg/ml LPS killed 60% of the p60−/− p80−/− cells. These results suggest that TNF receptors protect cells from LPS-induced cytotoxicity.

The cytotoxic effects of LPS in most cells are mediated through the activation of caspases, which degrade various substrates including PARP. To determine the effect of TNF receptors on LPS-induced caspase activation, the cells were treated with LPS at a concentration of 0.03 μg/ml for various times and then examined for PARP cleavage by Western blot analysis. LPS cleaved PARP in a dose-dependent manner in all cell types (Fig. 8B). The amount of 85-kDa protein gradually increased until the 24-h point, being highest in p80−/− and p60−/− p80−/− cells at 12 h. These results indicate that TNF receptor deletion also sensitizes the cells to LPS-induced caspase activation.

We also investigated the effect of TNF receptors on the LPS-induced apoptosis using annexin V staining. On treatment of wt, p60−/−, p80−/−, and p80−/− p60−/− cells with 0.1 μg/ml LPS for 12 h, annexin V-positive cells increased to 0.7, 15.3, 33.7, and 55.9%, respectively. Thus, the cells in which both receptors were deleted were maximally sensitive to LPS as indicated also by the annexin V staining assay (Fig. 8C).

Deletion of TNF Receptors Have No Effect on the Expression of CD14 and TLR4—Our results to this point indicated that deletion of TNF receptors sensitized macrophages to LPS-induced activation of NF-κB, IKK, JNK, p38MAPK, ERK1/2, and apoptosis. It was possible, however, that this sensitization was due to the up-regulation of LPS receptor induced by the deletion of TNF receptors, in an as yet undiscovered compensatory mechanism. Previous studies reported that LPS mediates its signaling through CD14 and TLR4 (7, 8). To determine the expression of CD14 and TLR4 in our TNF receptor-deleted macrophages, we prepared whole cell extracts and performed Western blot analysis using anti-CD14 and TLR4 antibodies. All of the macrophage cell lines expressed both CD14 and TLR4 (Fig. 9), and there were no significant differences in the expression of these proteins between different cell types. These results thus indicate that the difference in responsive of different cell types to LPS is independent of the expression levels of LPS receptors, CD14 and TLR4.
DISCUSSION

Gene deletion studies have shown that deletion of TNF receptor p60 or p80 induces resistance in mice to low levels of LPS. We investigated how LPS signaling is affected by the individual TNF receptors. We demonstrate that LPS-induced activation of NF-κB, IKK, JNK, p38 MAPK, and ERK1/2 were potentiated in macrophages in which the p60 TNF receptor or p80 TNF receptor or both receptors were deleted. Deletion of TNF receptors also enhanced the LPS-induced NO production, and iNOS and COX2 expression. LPS-induced apoptosis as indicated by cell viability, PARP cleavage, and annexin V staining was also increased in TNF receptor-deleted cells. The difference in LPS signaling between wt and TNF receptor-deleted cells was unrelated to the expression of the LPS receptors TLR4 and CD14. These studies indicate that deletion of either of the TNF receptors sensitizes the cells to LPS and suggests cross-talk between TNF and LPS signaling.

The deletion of p60 and p80 receptors had variable effects on LPS signaling. The deletion of p60 receptor had more pronounced effect than deletion of p80 receptor on LPS-induced NF-κB activation, whereas the reverse was the case for LPS-induced JNK activation, NO production, and for apoptosis. The precise basis for this differential effect is not clear.

How TNFR1 potentiates LPS-induced NF-κB activation is not clear. LPS activates NF-κB through sequential interaction with CD14, TLR4, MyD88, MyD88-adapter-like, IRAK-1 (IL-1 receptor-associated kinase-1), TRAF6, NIK (NF-κB-inducing kinase), and IKK, thus leading to NF-κB activation (15, 45). In contrast, TNF activates NF-κB through sequential interactions with TNFR1 and TNFR2 Deletion Sensitizes Cells to LPS Signaling

Fig. 6. Deletion of TNF receptors sensitizes macrophages to LPS-induced activation of p38 MAPK. One million wild type and TNF receptor-deleted cells were treated with 0.03 μg/ml of LPS for the indicated times. 30 μg of whole cell extract was fractionated on 10% SDS-PAGE and electrotransferred to a nitrocellulose membrane. Western blot analysis was performed using phospho-specific anti-p38 MAPK antibody as described under “Experimental Procedures.” The same membrane was rebolted with anti-p38 MAPK antibody.

Fig. 7. Deletion of TNF receptors sensitizes macrophages to LPS-induced activation of ERK1/2. One million wild type or TNF receptor-deleted cells were treated with 0.03 μg/ml of LPS for the indicated times. 30 μg of whole cell extract was fractionated on 10% SDS-PAGE and electrotransferred to a nitrocellulose membrane. Western blot analysis was performed using phospho-specific anti-ERK1/2 antibody as described under “Experimental Procedures.” The same membrane was rebolted with anti-ERK1/2 antibody.
CD14 or TLR4, because these receptors were expressed to equal extents in all cells. It is possible, however, that TNFR negatively regulates LPS-induced NF-κB activation by sequestering the LPS signaling proteins leading to NF-κB activation. For instance TNFR1 has a death domain that can potentially interact with other death domain-containing proteins such as MyD88 or IRAK-1. This possibility, however, seems unlikely because deletion of TNFR2, which does not have a death domain, was at least as effective as deletion of TNFR1 in potentiating the LPS-induced NF-κB activation. Another possibility is that LPS induces negative regulators of cell signaling that bind to TNF receptors such as SODD (silencer of death domain) (50).

We found that deletion of TNF receptors sensitized the cells to LPS-induced JNK, p38MAPK, and ERK1/2. The activation of JNK, p38MAPK, and ERK1/2 by LPS has been shown to require interaction with TRAF6, and that by TNF has been shown to require TRAF2. It is possible that enhanced sensitivity of cells to LPS was due to enhanced production of TNF resulting from higher NF-κB activation in TNFR-deleted cells. This is unlikely, however, because first the kinetics of NF-κB activation and MAPKs is comparable and second even if TNF is produced it would be nonfunctional because of a lack of TNFR.

We demonstrated that LPS-induced NO production and iNOS expression was enhanced in TNF receptor-deleted cells. The deletion of p80 receptor had a more pronounced effect on NO production than the deletion of p60 receptor (Fig. 3A). These results may explain why animals with deleted TNF receptors are protected from LPS-mediated toxicity (34–36). This protection may be provided by the higher levels of NO being produced. Additionally we found that LPS-induced COX2 expression was enhanced in TNF receptor-deleted cells, which may result in enhanced prostaglandin E2 production and protection of animals from LPS-mediated toxicity. We found that LPS-induced apoptosis was also enhanced by the deletion of TNF receptors. Interestingly, p80 receptor deletion (which lacks a death domain) was more effective than p60 receptor deletion (Fig. 8A) in enhancing LPS-induced cytotoxicity; deletion of both receptors was maximally effective. How TNF receptor deletion enhances the LPS-induced cytotoxicity is unclear. LPS has been shown to induce apoptosis in macrophages mostly through the autocrine production of TNF (32). This mechanism, however, is unlikely because if TNF is produced in an autocrine fashion, it would be nonfunctional without the TNF receptors.

The presence of the p60 receptor is required for resistance to Listeria monocytogenes, Mycobacterium tuberculosis, and Toxoplasma gondii (34, 36, 51, 52). Like our wt controls, TNF receptor-deleted mice were resistant to L. monocytogenes (35). However, in contrast to control mice, they were found to be resistant to TNF-induced skin necrosis (35). TNF signaling appears to be critical for protection against a large number of other infections by microorganisms (53–55). It is possible that some of these effects are mediated through cross-talk between TNF and LPS. Our results show that the deletion of TNF receptors makes the cells hypersensitive to LPS. Overall, our results clearly demonstrate that in macrophages, the deletion of either of the TNF receptors sensitizes the cells to the LPS-induced activation of NF-κB, JNK, p38MAPK, ERK1/2, and for the apoptosis.

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