A common regulatory locus affects both HNF4/HNF1α pathway activation and sensitivity to LPS-mediated apoptosis in rat hepatoma cells

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SUMMARY

Lipopolysaccharide (LPS) has been shown to protect certain cultured mammalian cells from undergoing programmed cell death (apoptosis) when exposed to tumor necrosis factor (TNF). However, LPS has also been reported to induce apoptosis in cultured endothelial cells, suggesting that apoptotic response mechanisms may be dependent upon cell type. In order to understand the influence of tissue-specific gene expression on apoptosis, we compared LPS-induced apoptosis in hepatoma cells with dedifferentiated hepatoma variant cells that have been selected for the loss of the liver-enriched HNF4/HNF1α transcriptional activation pathway. We report here that while human, rat and mouse hepatoma cell lines are resistant to LPS-mediated cell death, the HNF4+/HNF1α− rat hepatoma variant cells undergo rapid apoptosis (as determined by morphological analysis, DNA laddering and the TUNEL assay) upon exposure to LPS. Genetic rescue experiments show that restoration of the HNF4/HNF1α pathway via chromosome transfer render the hepatoma variant cells resistant to LPS-mediated apoptosis. However, the introduction of HNF1α alone failed to alter the apoptotic phenotype, suggesting that the defect(s) in the hepatoma variant cells that influence apoptotic responses lies upstream of HNF4/HNF1α expression. This study provides for the first time direct evidence of a common regulatory locus involved in activation of hepatic gene expression and sensitivity to LPS-mediated apoptosis.

Key words: Lipopolysaccharide, Apoptosis, Hepatoma

INTRODUCTION

Apoptosis plays a central role in development and homeostasis in metazoans (Jackson et al., 1997). Cellular machinery required to carry out apoptosis is resident in mammalian cells and can be activated by extracellular signals, often through ‘death receptors’ present on the cytoplasmic membrane. Activated death receptors (e.g. tumor necrosis factor receptor 1, TNFR1) interact with intracellular proteins resulting in the activation of caspases and cell death. Apoptotic machinery is kept in check by cellular proteins and it is the balance between survival and death signals that appears to dictate the response of cells to external stimuli (Ashkenazi and Dixit, 1998).

Lipopolysaccharide (LPS), a glycolipid component of the outer wall of Gram-negative bacteria, promotes the synthesis and release of tumor necrosis factor (TNF) and other cytokines that lead to septic shock (Beutler et al., 1985; Mathison et al., 1988). A proposed mechanism of LPS-induced endotoxic shock is through apoptosis of endothelial cells lining blood vessels. TNF release appears to play a primary role in septic shock. Interfering with TNF action using neutralizing antibodies or TNF binding proteins prevents endotoxic shock (Beutler et al., 1985; Lesslauer et al., 1991; Mathison et al., 1988). In addition, endotoxic shock is attenuated in mice lacking the TNF-55 receptor (Pfeffer et al., 1993; Rothe et al., 1993).

While TNF has been shown to induce apoptosis in a wide variety of tumor lines (Aggarwal and Natarajan, 1996), LPS-mediated apoptosis has been reported only in endothelial cells (Buchman et al., 1993; Choi et al., 1998; Meyrick et al., 1989). In other cell types, LPS has been reported to protect against apoptosis. Specifically, LPS reduced apoptosis caused by TGF-β in fetal hepatocytes (Martin-Sanz et al., 1996), TNFα in cultured lymphocytes (Manna and Aggarwal, 1999) and growth factor withdrawal in dendritic cells (Rescigno et al., 1998). Thus, cellular responses to LPS exposure appears to be dependent upon cell type.

We and others have previously established cell systems designed to study the regulatory pathways involved in establishing the hepatic cell phenotype. Dedifferentiated hepatoma variants cell lines have been derived that lack the major hepatic transcriptional activation pathway, HNF4/HNF1α, resulting in complete or partial silencing of a number of liver specific genes (Bulla, 1997b; Bulla and Fournier, 1994; Cereghini et al., 1988; Kuo et al., 1992; Spath and Weiss, 1997). HNF4, an orphan nuclear receptor, and HNF1α, a homeodomain protein, are highly expressed in the liver and expressed at reduced levels in other tissues such as kidney, intestine and pancreas (Duncan et al., 1994; Lazzaro et al., 1992).

We have utilized the dedifferentiated hepatoma variant cell lines to examine the influence of cell fate on sensitivity to LPS-mediated apoptosis. We report here that hepatoma variants undergo apoptosis when exposed to LPS, but the parental
hepatoma cells do not. Rescue of the hepatic phenotype in the variant cell lines with chromosome 12 reverses the sensitivity to LPS, but merely restoring of the HNF4/HNF1α pathway does not. Thus, these results suggest that regulatory factors upstream of the HNF4/HNF1α pathway are necessary for both establishing hepatic gene expression and protecting hepatoma cells from LPS-mediated apoptosis.

MATERIALS AND METHODS

Cell lines and culture conditions
Fado-2 is a rat hepatoma line lacking hypoxanthine-guanine phosphoribosyltransferase (HPRT) and adenine phosphoribosyltransferase (APRT) activities (Killary and Fournier, 1984). Fg-14 cells were derived from Fado-2 cells by two rounds of transfection; they contain stably integrated plasmids pAT-aprt and pAT-gpt, as described (Bulla and Fournier, 1992). Fg-14 cells were maintained in medium containing adenine-aminopterin-thymidine (AA T) to select for AT-aprt transgene expression. Hepatoma variant lines H11, M29, HS2, M38, M87 and M45 were derived from Fg-14 cells by selection in 2,6-diaminopurine (DAP) plus 6-thioxanthine (6-TX), and maintained in medium containing DAP, as described (Bulla and Fournier, 1992). HepG2 and Hep3B are human hepatoma cells obtained from American Type Tissue Culture. AT3Fh cells are mouse hepatoma cells (Antoine et al., 1992). All cell lines were maintained in 1:1 Ham’s F12:Dulbecco’s modified Eagle’s medium supplemented with penicillin, streptomycin, and 10% fetal bovine serum (Gibco). Cells and culture conditions

Reagents
Cycloheximide and LPS (Escherichia coli, 055:B5) were obtained from Sigma (St Louis, MO).

Viability analysis
Cells were treated with LPS alone, cycloheximide (CHX) alone or LPS plus CHX. At specific time points, cells were trypsinized and cell viability analysis was carried out by either trypan blue exclusion (microscopic examination) or propidium iodide uptake (FACS analysis).

Morphological analysis
Cells were trypsinized, centrifuged (600 g) for 10 minutes, resuspended with 100 μl PBS and then 1 volume of ice-cold methanol added dropwise. After 20 minutes, cells were centrifuged, resuspended in 3:1 methanol:acetic acid and applied onto microscope slides. Mounting buffer containing anti-fade (DABCO) plus 1 μg/ml Hoechst Dye was applied and cells were examined at ×400 and ×1000 magnifications using a Zeiss Axioplan fluorescent microscope. Cells exhibiting nuclear pyknosis, micronucleation, or chromatin blebbing were scored as apoptotic. Greater than 100 cells were scored with all treatments.

TUNEL assay
Tunel analysis was carried out using the Apoptosis Detection System from Promega, Inc. Briefly, treated cells were trypsinized, centrifuged at 600 g for 10 minutes, resuspended in PBS, applied to glass slides and allowed 20 minutes to attach. Cells were then fixed with 4% formaldehyde in PBS, incubated with a reaction mix containing fluorescein-labeled dUTP and terminal deoxynucleotidyl transferase for 1 hour, washed extensively and counter-stained with Hoechst dye. Positively stained fluorescein-labeled cells were visualized by fluorescence microscopy and compared to hoechst positive cells in order to determine the percent TUNEL positive cells.

DNA laddering assay
Cells grown on 60 mm dishes were lysed in 800 μl 0.6% sodium dodecyl sulfate, 10 mM EDTA, 10 mM Tris-HCl, pH 7.6. Detached cells were collected by centrifugation of cell supernatants at 600 g for 10 minutes, pellets were resuspended in 100 μl PBS and combined with the adherent cells. Protease (Sigma, St Louis, MO) and sodium chloride were added to cell lysates at final concentrations of 1 mg/ml and 0.8 M, respectively, and incubated overnight at 4°C. Samples were centrifuged 16,000 g for 10 minutes, and the supernatants transferred to fresh tubes followed by DNA precipitation with 2 volumes of ethanol. Samples were centrifuged for 10 minutes at 16,000 g and the DNA pellets resuspended in 20 μl water. Samples were treated with 2 mg/ml RNase A for 30 minutes at 37°C prior to electrophoresis on 1% Tris-borate agarose gels.

RNA analysis
RNA was extracted by NP-40 lysis and size fractionated on 1% agarose-2.2 M formaldehyde gels (Bulla et al., 1992). Cloned DNA sequences from α-tubulin (Kα-1) (Cowan et al., 1983) were labeled with [32P]dCTP by the random hexamer primer method (Feinberg and Vogelstein, 1983). The mouse α1AT probe is a 500-nucleotide [32P]UTP-labeled riboprobe from linearized pTS002.2 (Bulla and Fournier, 1994). To detect HNF4 expression, a 179 nt riboprobe (Bulla and Fournier, 1994) was used. For detection of HNF1α mRNA, a 254 bp fragment corresponding to exon 5 (nt 1867 to nt 2121) of the rat HNF1α cDNA was used, as previously described (Bulla, 1997a). Total cellular RNA (10 μg) was incubated with 1×10⁶ cpm of HNF4 and/or HNF1α riboprobe plus 2×10⁶ cpm of cyclophilin RNA riboprobe and incubated overnight at 52°C. The mixture was digested with RNase T1 +A, and protected fragments were resolved on 8% denaturing polyacrylamide gels. The gels were dried and exposed to film for 1 to 5 days.

RESULTS

Hepatoma variant cells undergo apoptosis in response to LPS
We investigated LPS-mediated cell death in a model system previously used to identify components responsible for hepatic gene expression. The dedifferentiated hepatoma variant cells were derived from Fado-2 rat hepatoma cells via a positive/negative selection scheme in which only cells which failed to activate a liver-specific promoter (the human α1-antitrypsin promoter) survived (Bulla and Fournier, 1992). The majority of the variant cells fail to express liver factors HNF4, HNF1α, or HNF4/HNF1α activated genes, including the endogenous α1-antitrypsin gene (Bulla, 1997b; Bulla and Fournier, 1994). Genetic rescue experiments showed that the hepatoma variants contain distinct defects, as introduction of HNF4 and/or HNF1α could fully rescue hepatic gene expression in only a subset of the clones (Bulla and Fournier, 1994).

Initial experiments showed that treating hepatoma variant cell lines with LPS for several days resulted in obvious cell death. In contrast, rat hepatoma cells were not noticeably affected by prolonged LPS exposure (data not shown). A previous report has shown that LPS-induced cell death of human endothelial cells requires the blocking of gene expression (Choi et al., 1998). We therefore incubated hepatoma variant cell lines with a combination of LPS plus cycloheximide, an inhibitor of protein synthesis. We first exposed hepatoma variant cell line M38 to increasing levels of LPS in the presence of 1, 5 and 50 μM cycloheximide for 14 hours. Cells were stained with propidium iodide (PI), which is

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excluded from the nuclei of intact cells, and dead cells were scored by FACS analysis. A striking level (68%) of cell death was observed within 14 hours in M38 cells exposed to LPS + CHx that was LPS-dose dependent (Fig. 1A).

We next monitored the time course of cell death in the M38 cells using 1 μg/ml LPS and 10 μg/ml CHx. We observed cell death (as scored by trypan blue exclusion) occurring by 4.5 hours of treatment with LPS + CHx. The percentage of M38 cells undergoing cell death increased dramatically at the 7 hour and 13 hour time points (Fig. 1B). Notably, treatment of the M38 cells with either LPS or CHx alone for 13 hours had little effect on cell death scored by this assay. Thus, LPS-mediated cell death in the M38 cells required inhibition of protein synthesis.

To determine whether the LPS-induced cell death observed in the experiments described above was unique to the M38 cells, we examined the effects of LPS + CHx on a panel of HNF4α-HNF1α− hepatoma variant cell lines (Bulla, 1997b; Bulla and Fournier, 1994). We monitored α1AT gene expression as an indicator of hepatic gene expression in these cells. The α1AT gene is regulated by HNF4 and HNF1α and therefore serves as a marker for expression of HNF4 and/or HNF1α (Bulla, 1997b; Bulla et al., 1998; Bulla and Fournier, 1994; De Simone et al., 1987; Li et al., 1988). As shown in Fig. 2A, each of the hepatoma variant cell lines fails to express the α1AT gene, whereas the hepatoma parental cells (Fg14) from which the hepatoma variant cells were derived express high levels of α1AT mRNA. One hepatoma variant line, M45, expressed low but detectable levels of α1AT mRNA.

Exposure of the panel of hepatoma variant cell lines (H11, M29...) with LPS + CHx resulted in cell death in each case, compared to minimal levels observed in the parental Fg14 hepatoma cells (Fig. 2B). In the majority of cell lines tested, neither LPS nor CHx alone induced cell death. Treatment of M45 and M87 cells with cycloheximide alone did result in an increase in cell death compared to the untreated cells (Fig. 2B). However, treatment with LPS + CHx resulted in much higher levels of cell death than did CHx alone in these latter cell lines.

We next asked whether LPS could induce apoptosis of other 'normal' hepatoma cell lines. Hepatoma cells derived from

Fig. 1. M38 hepatoma variant cells undergo cell death upon exposure to LPS + cycloheximide. (A) M38 Cells were treated for 14 hours with LPS (0 to 10 μg/ml) and cycloheximide (CHx) (1, 5, and 50 μM), stained with propidium iodide (PI) and PI uptake monitored by FACS analysis. (B) M38 cells were treated with LPS (1 μg/ml) plus CHx (10 μM) for 1.5, 2.5, 4.5, 7 and 13 hours and analyzed by trypan blue exclusion. As controls, the M38 cells were untreated or treated with LPS or CHx alone for 13 hours.

Fig. 2. Hepatoma variants, but not Fg14 cells, undergo LPS-mediated cell death. (A) Northern analysis of α1AT gene expression in Fg14 parental cells and hepatoma variant cell lines. The filter was reprobed with tubulin to control for RNA loading. (B) Fg14 hepatoma cells and hepatoma variant cells were untreated, treated with LPS (1 μg/ml) alone, CHx (10 μM) alone, or LPS + CHx for 14 hours and analyzed by trypan blue exclusion. All bars containing error bars represent results from triplicate independent experiments. Other values represent the average of two assays.
human (HepG2, Hep3B), rat (Fado-2, Fg-14) and mouse (AT3F) were tested. We first analyzed each hepatoma cell line for α1AT gene expression. Each of these cell lines express the α1AT gene (Fig. 3A). The HepG2 and AT3F cells appear to contain the highest levels of α1AT mRNA. However, the riboprobe used to detect α1AT is derived from a mouse α1AT cDNA, and thus may hybridize more efficiently with the mouse hepatoma cell line.

Treatment of hepatoma cell lines with LPS + CHx resulted in no significant cell death over the untreated controls (Fig. 3B). It was noted that certain hepatoma cells showed a modest degree of cell death during normal passaging of the cells. However, the addition of LPS or CHx to the hepatoma cell lines did not increase cell death. Also, RAT1 fibroblast cells were tested and found to be resistant to LPS-mediated cell death. Thus, the HNF4α/HNF1α− hepatoma variant cells appear to be uniquely sensitive to LPS-mediated cell death.

**LPS-induced cell death in hepatoma variant cells is consistent with apoptosis**

To determine whether the LPS-mediated cell death observed in the hepatoma variant cells was due to apoptotic mechanisms, we employed three assays commonly used to distinguish apoptosis from necrosis. First, we used fluorescent microscopy to examine nuclei stained with Hoechst dye. Apoptotic cells were scored on the basis of common characteristics of apoptosis: nuclear pyknosis, chromosome condensation, cytoplasmic blebbing, and chromatin patches in the nuclear periphery. In a panel of hepatoma and hepatoma variant cells, untreated cells showed only low levels of morphologically apoptotic cells. However, variant cells treated with LPS plus CHx showed levels of apoptosis (Fig. 4) that corresponded to cell death results as scored by trypan blue exclusion (Figs 2B and 3B). Thus, we observed general agreement between these two assays, suggesting that the trypan blue exclusion assay accurately reflects apoptosis occurring in the hepatoma variant cells.

We next carried out the TUNEL assay to verify that cells scored as apoptotic based on morphologic analysis would also meet the criteria of an independent assay. Fg14 and M38 cells were treated with LPS + CHx for 4.5 and 7 hours and analyzed. At the seven hour time point, 17% of M38 cells were found to be TUNEL positive, compared with 5% of Fg14 hepatoma cells (Fig. 5A and B). Attempts to score apoptosis in the M38 cultures at later time points were unsuccessful due the further breakdown of apoptotic cells to the point that cells failed to attach to the surface of the slides used in the detection assay. Because the cells were counterstained with Hoechst dye, we scored individual cells by both TUNEL and morphologic analysis. We conclude that the majority of cells scored as morphologically apoptotic are also positive by the TUNEL assay (results not shown). Thus, two independent assays appear to identify the same cells as being apoptotic.

For further verification of apoptosis in the hepatoma variant cells, we carried out the DNA laddering assay. Apoptosis is normally accompanied by cleavage of DNA between
nucleosomes, which produces a 180-200 bp DNA ladder and serves as an indicator of apoptosis (Jackson et al., 1997). M38 and Fg14 cells were treated with LPS plus CHx and DNA was isolated at 0, 3 and 7 hours post treatment. Equal amounts of DNA were electrophoresed on an agarose gel and stained with ethidium bromide. A high degree of DNA laddering was observed in the treated M38 cells but not the parental Fg14 hepatoma cells at the 7 hour time point (Fig. 6). This result is consistent with the hypothesis that apoptosis is occurring in the M38 cells in response to LPS + CHx.

**Restoration of hepatic gene expression renders hepatoma variants resistant to LPS-mediated apoptosis**

The above results suggest that the defects responsible for loss of hepatic gene expression in the hepatoma variants may also influence LPS-mediated apoptosis. To test this hypothesis, we analyzed clonal cell lines derived from hepatoma variant H11 cells in which the HNF4/HNF1α pathway has been rescued by the introduction of human chromosomes from human hepatoma HepG2 cells (Fig. 7A). These cell lines (designated H11(G2) series) were obtained from a large panel of H11 × HepG2 microcell hybrids which contain 1-3 human chromosomes (Bulla et al., 1998). We previously reported that only a small
subset of the microcell hybrid clones analyzed rescued hepatic gene expression, and that the rescuing locus mapped to human chromosome 12. Here we tested three H11(G2) cell lines that rescued hepatic gene expression and three cell lines that were nearly isogenic, but which failed to rescue hepatic gene expression (Bulla et al., 1998, and see Fig. 7A).

Treatment of the H11(G2) cells with LPS + CHx resulted in substantial apoptosis in parental H11 cells and non-rescued microcell hybrid lines H11(G2)-44, -52 and -81 compared with the untreated controls (Fig. 7B). In contrast, each of the microcell hybrid cell lines expressing the HNF4/HNF1α pathway, H11(G2)-71, -87 and -89, were resistant to LPS-mediated apoptosis. We noted that the H11(G2)-89 cells had a higher level of cell death in the untreated controls than did the other cell lines tested. However, there was no increase of cell death in the CHx + LPS treated cells relative to CHx alone, suggesting that the increase was due to CHx and not LPS-mediated apoptosis. Thus, these results suggest a direct correlation between hepatic gene expression and LPS-induced apoptosis.

**Ectopic HNF4/HNF1α expression fails to prevent LPS-mediated apoptosis**

The above results suggested that regulatory pathways responsible for hepatic gene expression influence the sensitivity to LPS-induced apoptosis observed in the hepatoma variant cell lines. In order to determine whether altered sensitivity to LPS-mediated apoptosis is influenced by HNF4, HNF1α, or HNF4/HNF1α responsive genes, we asked whether the introduction of an HNF1α expression plasmid into H11 cells could prevent LPS-induced apoptosis. We tested two clones, H11B1-1 and -16, which have been previously described (Bulla et al., 1998; Bulla and Fournier, 1994). These are H11 hepatoma variant cells that express a transfected HNF1α transgene resulting in restored expression of HNF4 as well as a number of additional hepatic-specific genes. To ensure that the H11 transfectants continue to express hepatic genes, we monitored α1AT gene expression, a gene whose expression is tightly correlated with HNF4/HNF1α expression in hepatoma cell lines (Bulla et al., 1998; Bulla and Fournier, 1994). As shown in Fig. 8 (right panel), α1AT mRNA levels are undetectable in the H11 cells, but fully rescued in the H11b1 clones compared to the hepatoma parental Fg14 cells. Treatment of the H11b1-1 and -16 clones with LPS + CHx resulted in levels of apoptosis similar to or higher than those observed in the H11 cells (Fig. 8, left panel). This suggests that the presence of the HNF4/ HNF1α pathway (or HNF4/HNF1α activated genes) in the H11 cells is not sufficient to confer resistance to LPS-induced apoptosis, and that factors upstream of this pathway are required for protection from LPS-induced apoptosis.

**DISCUSSION**

In this report, we have used a model system to identify a link between regulatory pathways responsible for cell fate and pathways involved in apoptosis. We consistently found that dedifferentiated hepatoma variant cells lacking HNF4/HNF1α expression were susceptible to LPS-mediated apoptosis. This result is in clear contrast to LPS-mediated effects observed in human, mouse and rat hepatoma cell lines. Restoration of the hepatic phenotype, by introducing human chromosomes into the variant cells, rendered the hepatoma variant cells resistant
expression and cellular responses to inflammatory agents. 

Fig. 8. HNF1α expression in H11 cells restores hepatic gene expression but does not affect LPS-mediated apoptosis. (A) An HNF1α expression plasmid was introduced into H11 cells. Clonal lines H11b1-1 and -16 have been previously shown to re-express HNF1α and HNF4 (Bulla and Fournier, 1994). (Right panel) α1AT levels were determined by northern analysis. Tubulin levels were monitored on the same filter to control for RNA loading. (Left panel) Cells were untreated, treated with LPS (1 µg/ml) alone, CHx (10 µM) alone, or LPS + CHx for 14 hours and analyzed by trypan blue exclusion. Results shown represent results from triplicate independent experiments.

Fig. 14. HNF4+/HNF1α expression plasmid was introduced into H11 cells. Clonal lines H11b1-1 and -16 have been previously shown to re-express HNF1α and HNF4. HNF4+/HNF1α-activated genes (including the α1AT gene). Thus, the observed LPS-mediated apoptosis appears to be independent of expression of the HNF4/HNF1α pathway itself, but rather is dependant on a regulatory event acting upstream of the HNF4/HNF1α pathway. We cannot rule out the possibility that these two phenotypes (LPS-mediated apoptosis and hepatic gene activation) are directed by distinct loci. However, the ability to both rescue the HNF4/HNF1α pathway and protect cells from LPS-induced apoptosis by the transfer of chromosomes from a human hepatoma cell line suggest that they share a common regulatory locus. Previous analyses of the hepatoma variants suggest that they contain distinct defects, based upon the ability to rescue HNF1α and/or α1AT expression by the introduction of trans-acting factors. However, this does not preclude the possibility that the same upstream regulatory locus is involved in each variant cell line and that this locus may also affect sensitivity to LPS-mediated apoptosis.

A proposed mechanism of endotoxic shock is through induction of apoptosis in endothelial cells lining blood vessels. Besides the LPS-mediated cell death reported in the hepatoma variant cells, endothelial cells are the only cells described that undergo LPS-mediated apoptosis. The fact that the hepatoma cell lines tested in our experiments fail to undergo LPS-mediated apoptosis suggests that the initiation of apoptotic responses in hepatic cells may be tied to programs responsible for maintaining tissue type.

The mechanism of LPS-mediated apoptosis is not well understood. LPS is considered to mediate septic shock, including apoptosis, by inducing expression of inflammatory cytokines like TNF. FADD, a protein involved in TNF signaling, has been reported to be involved in LPS-mediated signaling in endothelial cells which are sensitive to LPS-mediated apoptosis (Choi et al., 1993). In addition, mice lacking TRAF-6 expression exhibit defective LPS signaling (Lomaga et al., 1999). However, in other systems LPS has been found to protect cells from TNF mediated apoptosis and TGF beta mediated apoptosis (Manna and Aggarwal, 1999; Martin-Sanz et al., 1996). This protective effect of LPS has been suggested to be due to the activation of NF-κB, which is induced after exposure to LPS or TNF (Lee and Young, 1996; Manna and Aggarwal, 1999; Thompson et al., 1995). Preventing NF-κB activation accelerates TNF-mediated apoptosis in certain cell types (Beg and Baltimore, 1996; Liu et al., 1996; Van Antwerp et al., 1996; Wang et al., 1996). Finally, LPS has been shown to stimulate ceramide release (Haimovitz-Friedman et al., 1997) and induce the stress activated protein kinase (SAPK/JNK) pathway (Swantek et al., 1997; Westwick et al., 1994). The hepatoma variant system presented here provides an excellent opportunity to dissect the roles of these signaling pathways in LPS-mediated apoptosis and to identify regulatory genes that confer resistance to LPS-mediated apoptosis.

Two LPS receptors, CD-14 and Tlr-4, have been described. CD14 is primarily expressed on myeloid cells (Wright et al., 1990), and is able to confer LPS reactivity when introduced into CD14− cells (Gregner et al., 1995). In addition, over-expression of CD14 in transgenic mice resulted in LPS hypersensitivity (Yamamoto et al., 1998). The second LPS receptor described (Poltorak et al., 1998) is expressed primarily in lymphoid tissues (Medzhitov et al., 1997). It is unclear whether either of these receptors are present in hepatocytes or are required for LPS-mediated apoptosis observed in the dedifferentiated hepatoma variant cells.

It is possible that the susceptibility to LPS-mediated cell death observed in the hepatoma variant cells may reflect a mechanism of cell clearance, whereby cells that lose their ability to carry out appropriate cell-specific function are more susceptible to cell death induced by a LPS, cytokines or growth factors. It would be of interest to test this hypothesis by monitoring hepatic gene expression and apoptosis simultaneously in damaged livers.

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