Review

Hepatic mechanisms for clearance and detoxification of bacterial endotoxins

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Introduction

Endotoxins are components of the outer cell wall of most Gram-negative bacteria. Structurally they consist of a lipid component, lipid A, linked to a complex polysaccharide. Endotoxins are often referred to as lipopolysaccharides (LPS). This is a more purified form of endotoxin from which the membrane-associated proteins have been removed. The polysaccharide is divided into two regions, a core polysaccharide that is largely conserved among the Gram-negative bacteria and an outer region whose structure shows great variability between bacterial species. This outer polysaccharide is known as the O-antigen or O-specific polysaccharide. The detailed structure of many of the bacterial lipopolysaccharides are now known, and all follow this same basic structure. A generalized structure for the lipopolysaccharide of *Salmonella* is shown in *Figure 1*. A recent detailed discussion of endotoxin structure can be found in Ref. 1.

The Gram-negative flora of the terminal ileum and large intestine form a large reservoir of endotoxins. The concept that endotoxin could be absorbed from the gut was first demonstrated in models of hemorrhagic shock.[2] Using everted gut sacs and ^{51}Cr-labeled endotoxin, Nolan et al.[3] suggested that endotoxin does not traverse the gut as a passively permeable solute but is actively transported. These studies suggest that absorption of endotoxin from the gut and transport to the portal vein is a normal physiologic process. Furthermore, portal vein endotoxemia in normal subjects as well as significant systemic endotoxemia in patients with chronic liver disease has been demonstrated using the *Limulus* lysate assay.[4] These studies implicate the liver as the major organ involved in the clearance of gut-derived endotoxins preventing the occurrence of systemic endotoxemia. In patients with liver diseases, such as cirrhosis, impairment of liver function may lead to systemic endotoxemia[5] and intensify the underlying liver disease.

Endotoxin has been shown experimentally to be a necessary component in the pathogenesis of both chronic and acute liver disease. Broitman et al.[6] demonstrated that endotoxin is necessary for the development of the nutritionally induced choline-deficiency cirrhosis in rats. Adult rats fed a choline-deficient diet were protected from the development of cirrhosis but not hepatic steatosis by the addition of neomycin to their drinking water; however, addition of endotoxin (6 mg%) to the neomycin-containing drinking water overcame the protection afforded by antibiotic supplementation. This study was the first to suggest that hepatic disease may arise by inhibition of the liver's physiologic detoxification mechanism for normal flora endotoxins.

The presence of endotoxin is also required for the acute liver toxicity associated with exposure to carbon tetrachloride (CCl_4)[7] and D-galactosamine.[8] Impairment of the clearance of circulating endotoxins from peripheral circulation has been shown in CCl_4 toxicity,[9] while pretreatment with the antiendotoxin polymixin B protected animals from CCl_4 toxicity. However, gentamicin, which does not interact with endotoxin, has no protective effect.[10]

Similarly in rabbits, resection of the small bowel and colon eliminated the normal flora as a source of endotoxin and protected them from D-galactosamine hepatotoxicity.[11] D-Galactosamine acts directly upon the hepatocyte causing toxicity by depleting uridine nucleotides resulting in impairment of macromolecular synthesis[12] and may impair the liver's ability to deal with normally innocuous portal vein endotoxemia. The concept that hepatotoxin sensitivity correlates

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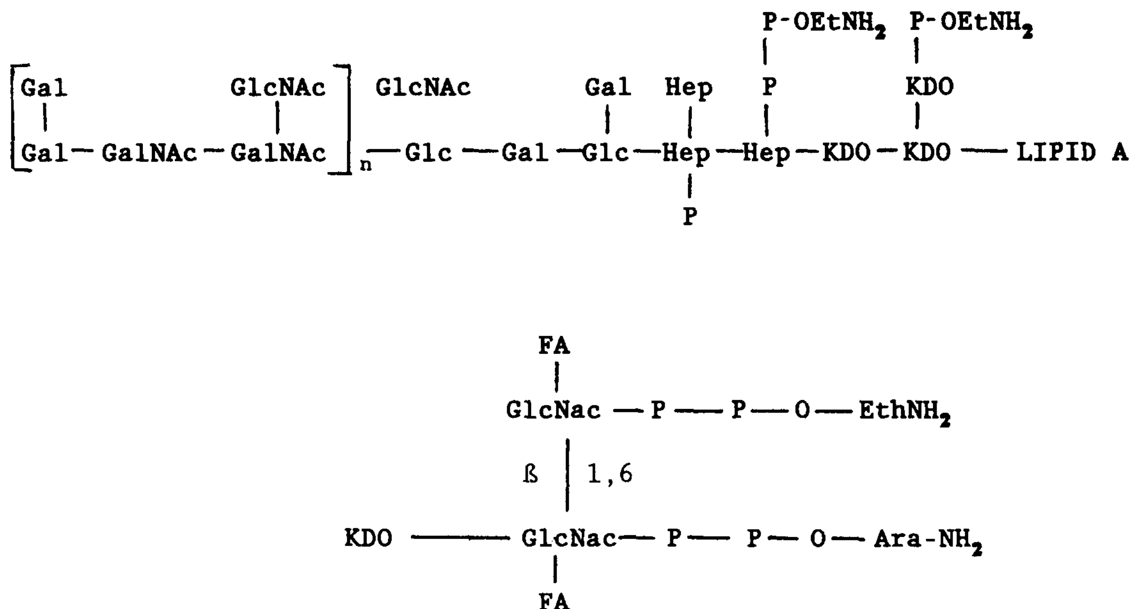

Figure 1 Generalized structure for the lipopolysaccharide from *Salmonella*. The structure comprises three regions: (1) The repeating oligosaccharide unit or O-antigen consisting of galactose (Gal) and N-acetyl glucosamine (GalNAc); (2) the inner core oligosaccharide containing the 3-deoxy-D-manno-octulosonic acid (KDO) and ethanolamine ($OEtNH_2$); (3) the lipid component, lipid A. The lower part of the diagram shows the lipid A structure in more detail, although the fatty acids (FA) are not represented. A detailed account of endotoxin structure can be found in Ref. 1.

with endotoxin sensitivity was demonstrated with the endotoxin-resistant mouse strain C3H/HeJ and the histocompatible, endotoxin-sensitive strain C3H/HeN. Unlike the HeN strain, HeJ mice were resistant to D-galactosamine–associated endotoxin lethality. Levels of serum alanine amino-transferase measured in galactosamine-treated resistant mice were only 10–30% of that of the sensitive strains. Adoptive transfer of macrophages from sensitive to lethally irradiated resistant strains transferred both endotoxin and D-galactosamine sensitivity.[13] Thus, the active cell in galactosamine-associated endotoxin toxicity is most likely a macrophage and from the above study most probably the Kupffer cell. Recent results suggest that D-galactosamine sensitization to endotoxin may be associated with exquisite sensitivity to cytokines.[14] In these experiments galactosamine-treated mice became sensitive to submicrogram quantities of tumor necrosis factor (TFN), one of the products of endotoxin sensitization of macrophages.[15] TNF lethality mimicked that of endotoxin in that it was reversible by the administration of uridine. However, TNF lethality was also observed in the HeJ endotoxin-resistant mice.

Endotoxin also plays an essential role in the pathogenesis of murine hepatitis induced by frog virus 3. This virus is known to target Kupffer cells with lethal consequences.[16] However, prior colectomy protects against the fatal effects of the virus.[17] The direct role of endotoxin in frog virus 3 hepatitis was shown when inoculation of a 1% lethal dose of endotoxin together with the virus negated the protective effects of colectomy.[16] Other studies have demonstrated that germ-free or C3H/HeJ mice are resistant to the virus and pretreatment of mice with polymyxin B significantly decreases the observed mortality and level of serum alanine aminotransferase.[18]

Recent studies have demonstrated that there exists an impairment of normal Kupffer cell function in liver disease. Studies of experimental alcoholic liver disease in rats showed that in Kupffer cells isolated from the diseased livers the lysozyme content is decreased.[19] Human monocytes also show both a decrease in lysozyme content and secretion when they were treated in vitro with ethanol.[20] Kupffer cells from chronic ethanol–fed rats demonstrate markedly depressed phagocytic capacity,[21] and several studies have suggested that Kupffer cells in the damaged liver can influence in vitro proliferation of both fibroblasts and fat-storing (Ito) cells. Proliferation of fat-storing cells increased when they were exposed to conditioned medium from Kupffer cells isolated from D-galactosamine or thioacetamide-injured livers.[22] Normal rat Kupffer cell–conditioned medium was shown to contain two factors (5 and 25 kDa) that inhibit in vitro proliferation of skin fibroblasts; however, the conditioned medium from Kupffer cells isolated 48 hours after administration of CCl_4 contained an additional 17 kDa factor that stimulated proliferation of both skin fibroblasts and fat-storing cells.[23] The proliferative factor was not Interleukin 1 (IL-1), a known stimulatory factor for fibroblasts,[24] and has yet to be fully characterized. Hepatic disease associated with Kupffer cell malfunction has also been demonstrated in a mouse model of viral hepatitis.[25] Mice fed a hypercholesterolemic diet had both impairment of Kupffer cell function and increased susceptibility to viral infection.

A role for endotoxin has also been shown in the development of lung diseases such as adult respiratory distress syndrome.[26] Endotoxin entry into the lungs causes pulmonary hypertension and microvascular permeability.[27] Similar to the effects seen in the liver, the interactions of macrophages with endotoxins are thought to play a role in the development of lung fibrosis by causing release of fibroblast growth regulators.[28] In both the lung and the liver, whose resident macrophage populations are chronically exposed to endotoxins, impairment of endotoxin-processing mechanisms can lead to pathological consequences that are often lethal.

Based on the above studies, it is reasonable to hypothesize that impairment of the physiologic mechanism for clearance and detoxification of endotoxin may lead to further development of either hepatic or possibly lung disease. These changes in tissue pathology may be induced either by endotoxin directly or by endotoxin-stimulation of immune cells, producing cytokines and lymphokines leading to localized immune responses. This article attempts to cover the present state of knowledge of the physiologic mechanism present in the liver for the clearance and detoxification of endotoxins.

The liver as clearance site

Accumulated evidence from a number of experimental studies has shown that in rodents, intravenously administered endotoxin is cleared from the systemic circulation principally by the liver. For example using an ^{125}I-labeled wild-type *Escherichia coli* or rough mutant *Salmonella minnesota* Re595 LPS, Mathison and Ulevitch[29] studied LPS clearance from the blood and subsequent tissue accumulation in rabbits. Clearance of both forms of LPS was biphasic, consisting of a rapid phase lasting fifteen minutes, in which the majority of the label was cleared and a slower phase lasting several hours. The majority of tissue-bound LPS was found in the liver with lesser amounts in the spleen, lung, kidney, adrenals, and muscle. Electronmicroscopic autoradiography showed the labeled LPS accumulated within Kupffer cells. In rats, LPS is cleared initially directly by the liver and later in association with high density lipoprotein.[30] A similar distribution was seen in rats using immunoperoxidase staining of the liver with LPS antibodies. LPS was first associated with Kupffer cells and after three days was detected in the hepatocytes.[31] Beyond three days LPS was no longer detectable by LPS antibody, suggesting that the molecule had been eliminated or that its antigenic sites had been modified, implying that this process occurs in the hepatocyte and not the Kupffer cell.

Ruiter et al.[32] provided more evidence for the direct role of the Kupffer cell in endotoxin clearance by differential isolation of liver cell populations after injection of radiolabeled endotoxins. This study demonstrated that the Kupffer cell fraction accumulated 100 times more label than the endothelial cell fraction and 1000 times more than the parenchymal cell fraction.[32]

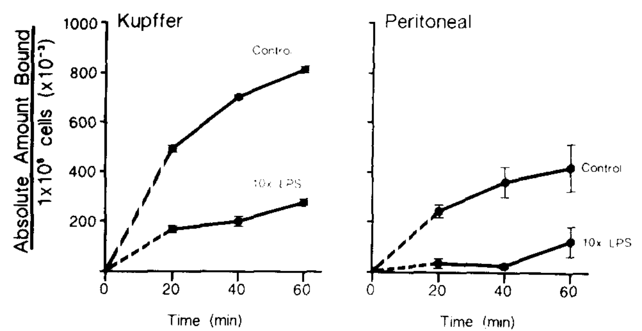

Figure 2 Uptake of ^{125}I lipopolysaccharide (LPS) by isolated rat Kupffer cells (KC) and peritoneal cells (PC). Kupffer cells or peritoneal macrophages were isolated and suspended at 3 × 10^6 cells/ml. ^{125}I LPS was added to suspension cultures to achieve a final concentration of 16.67 μg LPS/ml. At the indicated times points samples were filtered through Whatman GF/C filters and washed with ice cold saline (From Fox, E.S., Thomas, P., Broitman, S.A. [1987]. Comparative studies of endotoxin uptake by isolated rat Kupffer and peritoneal cells. *Infect. Immun.* **55,** 2962–2966, by permission).

Using a slightly different approach, Praaning-van Dalen et al.[33] studied liver reticuloendothelial system clearance of five radiolabeled test substances, polyvinylpyrrolidone, colloidal albumin, antimony sulfur colloid, endotoxin, and heparin. These results confirmed the previous study showing that endotoxin was exclusively taken up by Kupffer cells while the other substances were cleared by both parenchymal and endothelial cell populations. The Kupffer cell thus maintains an exquisite ability to clear endotoxins from the peripheral circulation and, presumably even more efficiently, gut-derived endotoxins from the portal vein. Its role in the detoxification process has not been proven and this role probably belongs to the hepatocyte.

Endotoxin-macrophage interactions

The Kupffer cell

The in vitro localization of intravenously injected endotoxin to the Kupffer cell initiated further studies using isolated macrophages. Direct studies of endotoxin uptake by isolated rat Kupffer cells indicated that these cells employ a mechanism of absorptive pinocytosis for internalization of bacterial endotoxins.[34] This mechanism is quite unlike that of isolated rat peritoneal macrophages that possess a saturable receptor meditated system for endotoxin endocytosis[35] (*Figure 2*). Using a photoactivatable, iodinatable crosslinker bound to endotoxin, Morrison and his colleagues[36] demonstrated an 80 kDa endotoxin specific binding protein on the surface of elicited rat peritoneal macrophages. A cell surface endotoxin binding protein of similar size and pI has also been detected on murine spleen cells and isolated spleen cell populations[37] and on peripheral blood mononuclear cells from endotoxin sensitive species.[38] Monoclonal antibodies to this protein can activate macrophages to become tumori-

cidal.[39] These studies suggest that this protein is an endotoxin receptor for immune cells and that the various effects of endotoxin stimulation may be manifested through its action.

With a similar endotoxin crosslinker complex, it was not possible to detect the 80 kDa endotoxin specific binding protein on the Kupffer cell surface while its presence was easily detected on elicited peritoneal macrophage from the same animals. The lack of specific endotoxin binding proteins is, however, unrelated to the presence of gut flora endotoxins, since Kupffer cells isolated from germ-free rats possess similar kinetics.[40] These results are consistent with the data from the in vitro studies of endotoxin uptake by isolated Kupffer cells that showed that Kupffer cells internalize endotoxin by nonspecific pinocytosis rather than receptor-mediated endocytosis.[35] Kupffer cell uptake of labeled rough strain LPS demonstrates that the rate of uptake is associated with the relative lipophilicity of the LPS micelle. The rate of uptake of wild-type LPS was increased in the presence of excess unlabeled rough (lipid rich) LPS, while the rate of uptake of rough strain LPS was decreased in the presence of excess unlabeled wild-type (polysaccharide rich) LPS.[40] The lack of a specific binding protein on the Kupffer cell surface accompanied by rapid internalization of the ligand suggests a unique relationship between the LPS micelle and the plasma membrane. Other studies have shown that both human and rat Kupffer cells isolated from biopsy specimens can internalize endotoxins, while neither endothelial cells nor fat-storing cells isolated from the same specimen could internalize endotoxin.[41] These results confirm previous in vivo studies[32,33] and are critical, since endothelial cells have a demonstrated phagocytic capacity.[42] This suggests that the Kuppfer cell and perhaps other tissue-fixed macrophages have a specialized cell membrane that allows binding of large amounts of endotoxin that may be internalized during rapid turnover of sections of the plasma membrane. Once internalized by the Kupffer cell, endotoxin is initially associated with phagosomes and later with lysosomes, where modification of the molecule presumably takes place.[43]

Hepatocytes

Direct studies of LPS uptake by isolated hepatocytes have provided mixed results. While in vivo clearance studies have suggested that hepatocytes are involved in direct clearance of rough strain LPS,[29] our own studies[40] would suggest that this is a result of a greater affinity of the Kupffer cell for these chemotypes leading to a more rapid passage to the hepatocyte. An in vitro study that analyzed binding exclusively has suggested that the hepatocyte possesses a protein receptor specific for the inner core region of the molecule.[44] Studies of uptake—the sum of binding plus internalization—have suggested that the uptake of wild-type is nonspecific[45] and that wild-type LPS is not internalized while rough strains are rapidly processed.[46] These results may be due to experimental design as most studies suggest that the role of the hepatocyte in the clearance mechanism is thought to be secondary to initial clearance and modification by the Kupffer cell.

Other macrophages

We have also examined endotoxin uptake by the fixed alveolar macrophages of the lung. These studies showed that endotoxin processing was similar to that of the Kupffer cell (unpublished observations). Because of anatomical considerations, the lung macrophage plays little part in the clearance from the circulation of gut-derived endotoxins; however, it becomes much more important when becteria or endotoxins enter the lungs from the airways. Impairment of the lung macrophages' ability to deal with endotoxins could, therefore, result in similar cell damage and fibrotic responses as those seen in liver disease.

Hepatic detoxification of endotoxin

Once endotoxin was cleared from the portal circulation by the liver, it was assumed that it was modified and that this resulted in detoxification. In support of this idea Farrar and Corwin[47] demonstrated that incubation of endotoxin with homogenized liver from guinea pigs effectively detoxified endotoxin based on its lethality to chick embryos. In vivo studies showed that multiple injections of CCl_4 compromised viability in guinea pigs after intravenous endotoxin injection, demonstrating that a functional liver was essential to the detoxification process. Removal of the spleen had no effect on guinea pig survival.[47]

More direct studies of modification and detoxification of endotoxin have been limited to in vivo studies of distribution after intravenous injection. A biosynthetically double-labeled *Salmonella abortus equi* LPS was used to study both clearance and excretion in rats.[48] This double-labeled LPS, which had ^3H incorporated into the lipid component and ^{14}C incorporated into the polysaccharide component, allowed researchers to trace the relative distribution of each portion of the molecule within a particular organ and its excretion in urine or stool. The double-labeled LPS was cleared from the circulation mainly by the liver and spleen. In the liver, staining with peroxidase-conjugated anti-LPS antibodies showed that LPS was initially associated with Kupffer cells and only later with the parenchymal cells. Isolation of LPS from liver and spleen over 72 hours revealed a sequential loss of ^3H in comparison to ^{14}C, suggesting modification of the lipid component. The majority of radioactivity was found in stool with similar loss of ^3H. These studies suggested that the liver modifies endotoxin by deacylation. This was confirmed by reisolating LPS by aqueous phenol extraction, from whole liver following intravenous injection.[49] While the preparation was contaminated with liver-derived substances, biochemical analysis revealed a significant decrease in the

amounts of 3-hydroxytetradecanoic, hexadecanoic, and dodecanoic fatty acids. The liver-processed LPS was still reactive in the *Limulus* assay, equally toxic as native endotoxin to galactosamine-sensitized mice, and produced a Schwartzmann reaction in the same range as native endotoxin. Thus, while the liver uptake results in modification of the structure of endotoxin, no obvious detoxification was observed.

Kupffer cells

Experiments with isolated rat liver Kupffer cells and an LPS with biosynthetically labeled lipids (^{14}C) and sugars (^{3}H) suggested quite a different scheme for LPS processing.[50] These studies analyzed modification of ^{3}H-^{14}C-endotoxin by pulse chase experiments, changes in buoyant density in cesium chloride (CsCl) isopyknic density gradients, and analysis of micelle size by column chromatography. Unlike peritoneal cells, the Kupffer cell modified LPS preferentially within its polysaccharide component (*Figure 3*). Almost 80% of internalized polysaccharide and only 40% of lipid was released from the labeled-LPS over 18 hours. Preferential modification of polysaccharide and retention of lipid were consistent in studies of buoyant density and micelle size. Activity retained by Kupffer cells equilibrated at the least dense region of the gradient and showed a larger micelle. Processed Kupffer cell was still reactive in the chromogenic *Limulus* assay. However, processing did result in an increased rate of endocytosis of the Kupffer cell exocytosed endotoxin by the hepatocyte that could not be inhibited with excess asialo-fetuin, showing that this mechanism was not mediated by the hepatic galactose binding lectin[51] (unpublished).

Hepatocytes

Studies of modification of bacterial LPS by isolated hepatocytes have demonstrated that in vitro these cells deacylate LPS.[46] The key finding of this study was that in vitro hepatocytes cannot internalize wild-type, smooth strain LPS but are able to endocytose rough strain, deglycosylated LPS. Within the liver, clearance and modification of gut-derived bacterial endotoxin require multiple cell involvement; initial clearance and deglycosylation by the Kupffer cell were followed by deacylation and detoxification by the hepatocyte. This scheme is similar to that previously described for clearance and metabolism of carcinoembryonic antigen (CEA)[52] and the hemoglobin/haptoglobin complex.[53] In the case of CEA processing initial uptake by Kupffer cells is followed by exocytosis of CEA modified by loss of sialic acid. The asialo CEA is endocytosed by the hepatocyte asialoglycoprotein receptor and degraded.[53] This process of initial Kupffer cell clearance followed by passage to the hepatocyte may represent a general biochemical mechanism present in the liver for clearance and sequestration of gut-derived antigens.

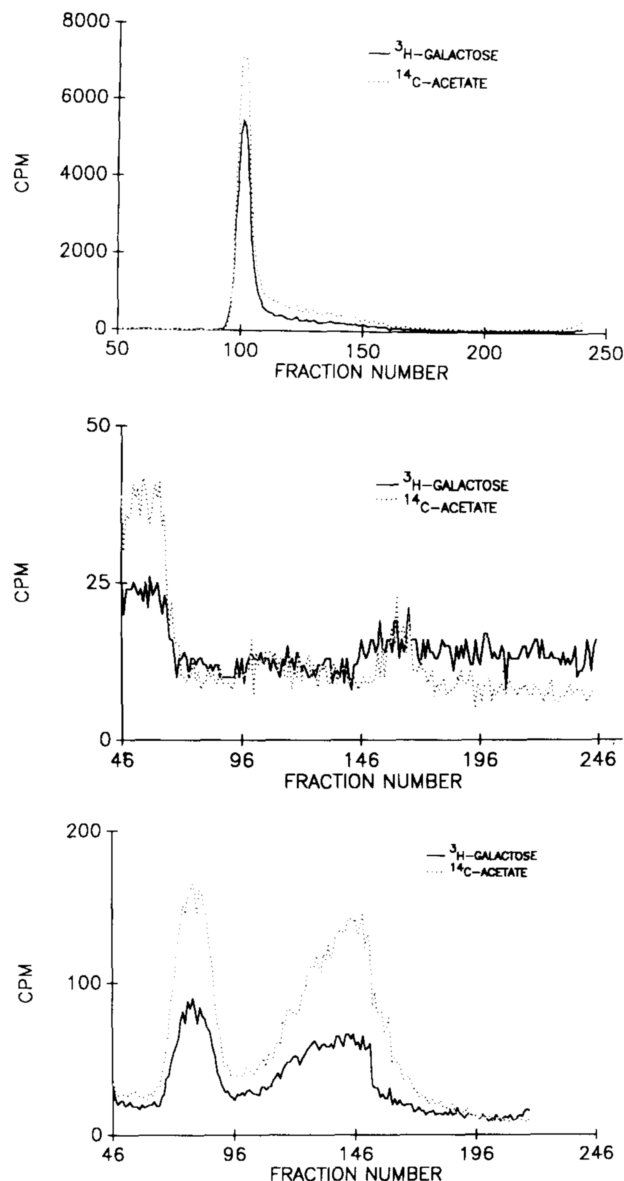

Figure 3 Kupffer cell modification of ^{3}H,^{14}C lipopolysaccharide (LPS). *Escherichia coli* J5 was biosynthetically labeled with ^{3}H and ^{14}C, and LPS was isolated by phenol-water extraction. Kupffer cells were isolated and suspension cultures established at 3×10^{6} cells/ml. Double-labeled LPS was added to suspension cultures and incubated for 1 hour at 37°C, after which cells were washed to remove unbound label. Cell suspensions were established at 1×10^{6}/ml and incubated overnight. After incubation cells were pelleted, lysed, and sonicated. Supernatants were concentrated by Amicon filtration. Micelle size was analyzed by column chromatography using Biogel A1.5. *Top*, native LPS; *center*, retained LPS; *bottom*, released LPS (From Fox, E.S., Thomas, P., Broitman, S.A. [1989]. Clearance of gut derived endotoxins by the liver: Release and modification of ^{3}H,^{14}C-lipopolysaccharide by isolated rat Kupffer cells. *Gastroenterology* **96,** 456–461, by permission)

Other macrophages

In vitro studies have examined endotoxin modification and detoxification in mouse peritoneal cells. The galactose epimerase deficient *E. coli* J5 mutant has been used to create a triple-labeled organism such that ^{3}H was specifically incorporated into LPS, uniformly la-

beled with ^{14}C, and other cell surface components labeled with ^{125}I (Ref. 54). By measuring the rate of release of the ^{3}H label after phagocytosis of the labeled *E. coli,* the fate of the LPS relative to other bacterial components could be followed. While over 90% of the surface components (^{125}I) and 80% of uniformly labeled (^{14}C) components were released by the cells, only 40% of the LPS (^{3}H) entered the medium. These results indicated that post-phagocytosis peritoneal cells catabolized *E. coli* but selectively retained the LPS component. Post-phagocytosis modification of LPS by peritoneal cells was determined by its characteristic buoyant density, relative to the native molecule, in CsCl isopyknic density gradients. Density gradients of retained or released activity showed that retained LPS was of a higher density than released LPS, indicating modification of the molecule. Native LPS was more dense than either cell-associated or released LPS. The peritoneal macrophage processed LPS was as toxic and more immunostimulatory than the native molecule.[55] It produced a lethal response in actinomycin D-treated mice and was capable of clotting *Limulus* lysates. It was also 10 to 100 times more effective than the native molecule in its ability to stimulate IL-1 production or proliferation of murine splenocyte cultures.

Structural aspects of peritoneal cell modification of LPS have been studied with *Salmonella typhimurium* LPS labeled with ^{3}H in its fatty acids and a ^{14}C in its polysaccharide.[56] Pulse chase experiments studying the rate of appearance of the ^{3}H label over the course of 72 hours suggested that modification takes place in the fatty acid portion of the molecule. Thin-layer chromatographic analysis of extracted fatty acids revealed that LPS recovered from cells was depleted in nonhydroxylated fatty acids. High-performance liquid chromatographic analysis revealed that deacylated LPS was enriched in 3-hydroxy-tetradecanoic acid and depleted in dodecanoic, tetradecanoic, and hexadecanoic acids. This is in direct contrast to Kupffer cell modification of LPS, where modification occurred primarily in the polysaccharide component and seemed to occur much more rapidly.[50]

Recent reports have demonstrated that toxicity and bioreactivity of LPS preparations are dependent on the degree of phosphorylation of the lipid A region.[57] A macrophage's ability to dephosphorylate LPS could be a mechanism for detoxification. Isolated peritoneal macrophages modified *E. coli* LPS labeled with ^{32}P and ^{3}H by dephosphorylation and deacylation.[58] This modified LPS was not examined for toxicity, though studies of chemically synthesized LPS made without the phosphate or acyl groups have shown that these modifications result in loss of toxicity. Catabolism of whole *E. coli,* however, did not demonstrate detoxification of endotoxin by peritoneal cells.[54,55]

Actual detoxification of bacterial endotoxin has only been shown by the action of human neutrophils.[59] Neutrophils modify endotoxin by selectively removing the nonhydroxylated fatty acids from the lipid A region. Partial purification of the acyloxyacyl hydrolase activity from promyelocytes and incubation with LPS in vitro have shown that deacylated LPS did not produce a Shwartzman reaction, and was not lethal to chick embryos while it still provided a proliferative stimulation to isolated B cells. These data suggest that different parts of the LPS molecule are responsible for the toxicity and proliferative effects respectively.

Kupffer cell: hepatocyte communication

Communication between Kupffer cells and hepatocytes has been shown in the processing of labeled endotoxins, carcinoembryonic antigen, and hemoglobin/haptoglobin complex.[31,52,53] In vitro co-culture systems have shown that the Kupffer cell may regulate hepatocyte biochemistry either through prostaglandin production or through synthesis of soluble factors de novo. For example, Kupffer cells co-cultured with hepatocytes produce a 25-30 kDa factor that stimulates fibrinogen synthesis five- to eightfold.[60] Other studies have implicated the Kupffer cell as the source of prostaglandin D_2 (Ref. 61) as well as E_2 (Ref. 62). These prostaglandins, produced in response to endotoxin stimulation, have been implicated in the regulation of protein production and glucose metabolism associated with sepsis.[63] Kupffer cell–produced prostaglandin has also been connected with the autoregulation of IL-1 production[64] and regulation of natural killer cell activity in vitro.[65]

Endotoxin also affects the oxidation of L-arginine to nitrite and nitrate. In vitro stimulation of murine macrophages with endotoxin and interferon gamma leads to the production of nitrate, nitrite, and citrulline from L-arginine.[66] Miwa et al. have shown that stimulation of macrophages by endotoxin results in the production of nitrite that, in turn, nitrosates amines to form nitrosamines, potent carcinogens.[67] Stimulation of isolated Kupffer cells with multiple cytokines prior to co-culture with hepatocytes leads to suppression of hypatocyte protein synthesis, which has been associated with metabolism of L-arginine.[68] These studies have not addressed the possible role of endotoxin or Kupffer cell–metabolized endotoxin in this process.

Gene expression and regulation by bacterial endotoxins

Identification of Kupffer cell genes responsive to the chronic endotoxemia of the portal vein has yet to be accomplished; however, LPS has long been used in vitro as a stimulator of cytokine synthesis and cellular differentiation for immune cells and macrophages. LPS is also capable of both up- and downregulation of macrophage surface markers such as Mac-1, -2, -3 and Ia.[69] Furthermore, mRNAs for colony stimulating factor-1, c-fos, IL-1, and TNF are induced on treatment of macrophages with LPS.[70] Most of the knowledge of endotoxin-regulated gene transcription has come from examining the expression of the cytokines IL-1 and TNF. Both proteins are synthesized by macrophages and monocytes de novo in response to endotoxin stim-

ulation. Induction of the IL-1 beta gene in the human monocytic leukemia cell line THP-1 in response to LPS stimulation was rapid, but expression was transient.[71] This study suggested that transcription of IL-1 beta was ultimately regulated by a transcriptional repressor protein as inhibition of protein synthesis with cycloheximide resulted in superinduction of the IL-1 beta gene. Transcription of IL-1 beta or IL-1 alpha can be modulated during exposure of a human monocytic cell line (U937) to interferon gamma.[72] Interferon treatment prolongs the expression of either gene fivefold. Both the IL-1 alpha and beta genes are expressed by isolated rat Kupffer cells. Northern blot analysis demonstrated differences in the abundance of these messages in either stimulated or unstimulated cells (unpublished observations). These results illustrate the pleiotropic aspects of endotoxin gene induction and regulation.

Regulation of the induction and transcription of the TNF gene has been extended to the sequence level and suggests a post-transcriptional mechanism. TNF is one of the major secreted products of endotoxin-stimulated macrophages. Stimulation results in a 100-fold increase in cellular mRNA.[73] Some authors[73] suggest that endotoxin stimulation results in as much as a 10,000-fold increase in protein synthesis and secretion. While initial exposure to endotoxin has profound effects on macrophage physiology, these same cells are refractory to a secondary exposure;[74] however, the refractory state is reversible. Tolerance to a second endotoxin exposure has been attributed to the macrophage's ability to internalize and not re-express its TNF receptor.[75] While there is no TNF release during the refractory state, transcription continues, as does translation of the 26 kDa TNF precursor protein.[76]

At the sequence level, transcriptional enhancement of endotoxin-mediated TNF transcription has been assigned to the presence of enhancing elements within the TNF gene.[73] The same family of enhancing elements are also found in genes for immunoglobiulins, Class I MHC, IL-2, various cytokines, and viruses. The translational level control of TNF has been linked to the presence of 3′ untranslated region (TTATTTAT).[77] The role of this sequence in mRNA degradation or translational efficiency has been investigated by creating deletion-bearing TNF genes to the chloramphenicol transacetylase gene. Such fusions were able to show that constructs that include the "regulatory" untranslated sequence were not translated. Such translational blockade has been demonstrated with IFN-β and colony stimulating factor.[78]

Recently, several other endotoxin responsive genes in peritoneal macrophages and macrophagelike cell lines have been identified, though the specific gene functions are unknown. Differential hybridization of cDNA from LPS-stimulated and -unstimulated murine peritoneal macrophages identified six genes that could be induced by LPS treatment and were not TNF, IL-1, or other known competence genes.[79] Four of the six genes were inducible by treatment with gamma interferon, macrophage colony stimulating factor, or phorbol myristate acetate; however, two were exclusively LPS responsive. Beyond the initial stimulatory event, transcription of these endotoxin responsive mRNAs was regulated by the intracellular cAMP level.[80] Three of these genes were also expressed by Balb/c 3T3 cells when stimulated by platelet-derived growth factor,[81] as well as murine Kupffer cells stimulated with endotoxin (unpublished observations), suggesting that these three peritoneal macrophage endotoxin responsive genes are not macrophage specific.

As well as inducing new gene expression, it is equally likely that exposure to endotoxin can downregulate genes; for example, Yurochko et al.[69] have shown decreases in the surface markers Mac-3 and Ia after exposing macrophages to LPS for 24 hours. Furthermore, evidence exists from functional studies that indicate that endotoxin exposure results in downregulation of normal gene expression. In a model of Kupffer cell suppression of hepatocyte protein synthesis Billiar et al.[82] showed that Kupffer cells from germ-free rats do not suppress protein synthesis without prior stimulation with endotoxin or colonization with Gram-negative flora. Treatment of mice with endotoxin decreases the hepatic clearance of circulating immune complexes and may be involved with the pathogenesis of systemic lupus erythematosus.[83] The authors suggest that this decreased clearance capacity stems from interference with Fc receptor function that could possibly involve downregulation of the gene. There are no direct studies to date, however, on the effects of endotoxin—neither the chronic portal endotoxemia nor systemic endotoxemia—on Kupffer cell gene expression.

Conclusions

It is likely from the functional studies described above that Kupffer cells with their unique mechanism for endotoxin processing and their position within the chronic endotoxemia of the portal circulation function differently from less differentiated macrophage populations. Modification of endotoxin and reexpression of the modified product into the sinusoid, where it can interact with other liver cells, may have an important bearing on the role that endotoxin plays in the etiology of liver disease. Future studies will define the relationship between endotoxin and its metabolites and cytokine production in the liver and their relationship to normal liver function and the development of disease.

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