Nonalcoholic Steatosis and Steatohepatitis
IV. Nonalcoholic fatty liver disease abnormalities in macrophage function and cytokines

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Diehl, Anna Mae. Nonalcoholic Steatosis and Steatohepatitis. IV. Nonalcoholic fatty liver disease abnormalities in macrophage function and cytokines. Am J Physiol Gastrointest Liver Physiol 282: G1–G5, 2002; 10.1152/ajpgi.00384.2001.—Macrophage products, such as cytokines, prostanoids, nitric oxide, and reactive oxygen intermediates, influence the function and viability of macrophages and neighboring cells. Given that the liver has one of the largest resident macrophage populations in the body, it is not surprising that hepatic macrophages [i.e., Kupffer cells (KC)] are involved in the pathogenesis of many kinds of liver disease. This review summarizes the abnormalities that have been demonstrated in bone marrow, peritoneal and hepatic macrophage of leptin-resistant (fa/fa) rats and leptin-deficient (ob/ob) mice, two animal models for nonalcoholic fatty liver disease (NAFLD). Evidence supports the concept that altered KC function influences the viability of other cells, such as lymphocytes and hepatocytes, in fatty livers, thereby contributing to the pathogenesis of NAFLD in animals with reduced leptin activity. Further work is needed to determine whether KC dysfunction is a component of more generalized mechanisms that lead to NAFLD.

steatohepatitis; innate immunity; leptin

Compared with other organs, the liver has one of the largest resident populations of macrophages, which are key components of the innate immune system. Resident hepatic macrophages, i.e., Kupffer cells (KC), are derived from circulating monocytes that arise from bone marrow progenitors. Once localized within the liver, these cells differentiate to perform specialized functions, including phagocytosis, antigen processing, and presentation. KC also generate various products, including cytokines, prostanoids, nitric oxide, and reactive oxygen intermediates. These factors regulate not only the phenotypes of the KC that produce them, but also the phenotypes of neighboring cells, such as hepatocytes, stellate cells, endothelial cells, and other immune cells that travel through the liver (15). Therefore, KC are intimately involved in the liver’s response to infection, toxins, transient ischemia, and various other stresses. Several laboratories have shown that KC contribute to the pathogenesis of many different kinds of liver injury (5, 31, 33, 35, 39). However, much remains to be learned about the environmental factors and genes that modulate KC behavior. This knowledge, in turn, will help to clarify how KC regulate the viability and function of other cells in the liver. Consequently, a better understanding of these issues should enhance the development of treatments for liver diseases that result from altered KC function.

This review will summarize recent data that suggest that KC dysfunction contributes to the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Most of this information has been generated by studying leptin-deficient (ob/ob) mice, which develop fatty livers spontaneously and naturally exhibit features such as insulin resistance, obesity, and dyslipidemia (27), that have been strongly correlated with NAFLD in humans (21, 22). The complex phenotype of ob/ob mice results from a spontaneous mutation in the ob gene that prevents the synthesis of leptin, an adipocyte hormone (27). Although initially identified as a satiety factor that inhibits feeding behavior by acting on hypothalamic neurons (10), leptin is now known to have potent immunomodulatory actions (11, 13, 19, 20, 32, 37). Because leptin is produced by adipocytes, serum leptin levels are actually increased in most obese, insulin-resistant humans with NAFLD. On the other hand, decreased leptin levels occur in humans with lipoatrophy, who also have a high incidence of insulin resistance and NAFLD (30). Given the variability of serum leptin concentrations in humans with NAFLD, it remains to be determined whether or not the KC and

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cytokine abnormalities that have been noted in ob/ob mice occur generally or in only specific subsets of humans with NAFLD. Nevertheless, the ob/ob data provide a useful starting point to guide future studies that seek to evaluate the role of KC in humans and other animal models of NAFLD as well as related liver diseases, particularly alcohol-induced liver injury.

ABNORMAL MACROPHAGE PHENOTYPE IN RODENTS WITH DECREASED LEPTIN ACTIVITY

Studies in obese, Zucker diabetic (fa/fa) rats provided the first clue that KC dysfunction might be involved in the pathogenesis of NAFLD (40). fa/fa Rats have a mutation in the long form of the leptin receptor (obRb) that inhibits leptin-initiated signal transduction (28). Hence, although serum concentrations of leptin are increased in fa/fa rats, these animals resemble ob/ob mice, which are genetically deficient in leptin. Both fa/fa rats and ob/ob mice are obese, insulin-resistant, develop fatty livers, and are unusually vulnerable to liver injury induced by bacterial lipopolysaccharide (LPS). In both strains, a typically innocuous dose of LPS induces severe fatty liver hepatitis (i.e., steatohepatitis) (40). Therefore, these animals provide models that can be used to probe the mechanisms that lead to the development of this type of liver injury.

Extensive work by many groups has proven that LPS-liver injury is mediated by tumor necrosis factor-α (TNF-α), in normal rats and mice. Factors, such as interleukin (IL)-12 and -18 and interferon (IFN), that enhance TNF-α activity generally exacerbate LPS liver injury, whereas those that inhibit TNF-α, such as IL-10, are hepatoprotective (24). Although many types of cells are capable of producing TNF-α, KC are thought to be the principal hepatic source of TNF-α after LPS administration, because LPS is a potent KC activator, and various treatments that inhibit KC activation protect rats and mice from LPS-liver injury (34). Baseline serum concentrations of TNF-α as well as TNF-α gene expression in liver and white adipose tissue are slightly, but significantly, increased in fa/fa rats and ob/ob mice compared with their lean littersmates. However, TNF-α expression is not induced supranormally following LPS administration in either strain (40). Therefore, enhanced sensitivity to TNF-α hepatotoxicity, rather than overproduction of TNF-α, must underlie the increased liver damage that occurs in fa/fa rats and ob/ob mice compared with their littermate controls after LPS treatment.

Because KC are major producers of the cytokines that modulate TNF-α activity, it was logical to screen fa/fa rats and ob/ob mice for evidence of KC alterations. Analysis of the pre- and post-LPS liver samples demonstrated significantly and consistently reduced expression of KCR, a KC-specific gene, in fa/fa livers. In contrast, KCR expression decreased only transiently after LPS administration in control animals. Moreover, fa/fa rats exhibited reduced hepatic clearance of intraperitoneally administered fluorescent-labeled microspheres. Neither of these deficiencies in fa/fa KC function was likely to have been the result of decreased numbers of hepatic macrophages, because the hepatic expression of Pu-1, a myeloid enriched transcription factor that can be used to approximate KC number, was similar in fa/fa rats and controls (40). Further support for the possibility that the phenotype of KC is abnormal in the LPS-sensitive fa/fa rats was provided by subsequent experiments that compared the phagocytic function and cytokine production of cultured peritoneal macrophages and bone marrow cells from control rats and mice with those of ob/ob mice, fa/fa rats, and db/db mice (which also have dysfunctional leptin receptors). Macrophages derived from all of the strains with decreased endogenous leptin activity exhibited reduced phagocytic activity in vitro. In cells with functional leptin receptors, this defect was improved by adding leptin to the culture medium (19). Thus chronic inhibition of leptin signaling reversibly reduces the phagocytic function of macrophages. Macrophages from strains with reduced leptin activity also demonstrated other abnormalities, such as abnormal cytokine production basally and/or after challenge with LPS. For example, LPS induction of IL-6 was enhanced significantly, whereas that of IL-10 was inhibited in fa/fa or ob/ob cells. However, unlike phagocytosis, overnight incubation in leptin-containing medium did little to correct these abnormalities (19).

MECHANISM FOR MACROPHAGE ABNORMALITIES ASSOCIATED WITH LEPTIN INSUFFICIENCY

Certain “baseline” features of hepatic and/or peritoneal macrophages from fa/fa rats and ob/ob mice (e.g., decreased KCR expression, increased IL-6 production) resemble those that occur in macrophages from normal animals following LPS administration. LPS is known to induce reactive oxygen intermediates, such as H2O2, and activate redox-sensitive transcription factors, such as nuclear factor (NF)-κB and CCAAT enhancer binding protein (C/EBP) β, that upregulate cytokine gene expression in macrophages (24). Therefore, the possibility that macrophage oxidant production might be increased in ob/ob macrophages was evaluated. Although membrane-associated NADPH oxidase is an acknowledged source of oxidants in myeloid cells, our studies focused on macrophage mitochondrial oxidant production, a process that can be regulated by mitochondrial uncoupling protein (UCP)-2. In normal rodents, KC express UCP-2 constitutively (14), and factors that inhibit UCP-2 increase macrophage H2O2 production (25). Interestingly, in vitro exposure to LPS quickly downregulated UCP-2 mRNA expression in normal peritoneal macrophages. This was followed by increased macrophage production of superoxide anion and H2O2, the induction of NF-κB and C/EBP β, increased expression of IL-6, a cytokine gene that is activated by these transcription factors, induction of cyclooxygenase (COX)-2, an IL-6 inducible enzyme, and increased production of the COX-2 product, PGE2. Steady-state levels of UCP-2 mRNA were reduced in ob/ob peritoneal macrophages both before and after LPS treatment, and this was associated with signifi-
cantly increased oxidant generation and amplification of all of the ensuing redox-regulated events that culminated in markedly increased production of PGE₂ (17). If similar responses occur in hepatic macrophages, the enhanced prostanoid production might drive some of the changes, such as the increased expression of bcl-2 family members, that have been noted in fatty ob/ob hepatocytes (29). The latter possibility is supported by recent evidence that increased KC COX-2 activity upregulates hepatocyte bcl-2 expression in an animal model of cholestasis (35).

At this point, it is uncertain whether the altered phenotype of ob/ob macrophages actually results from endogenous exposure to low levels of LPS or to some direct (or indirect) effect of leptin deficiency on macrophage differentiation. Although there is, as yet, no proof of increased systemic or portal endotoxemia in ob/ob mice, evidence demonstrates that aging ob/ob mice develop intestinal stasis, bacterial overgrowth, and enhanced exposure to ethanol and other products of intestinal bacteria (3). Thus there is some support for the first hypothesis. Nevertheless, a more direct role for leptin deficiency on macrophage differentiation also merits consideration, because bone marrow progenitor cells are known to express obRb, and leptin has been shown to regulate both myeloid and erythroid differentiation (13, 37).

HEPATIC CONSEQUENCES OF KC ABNORMALITIES IN OB/OB MICE

As mentioned earlier, KC elaborate products that regulate the viability and function of neighboring cells. Although macrophage-derived PGE₂ can induce anti-apoptotic proteins in hepatocytes, other macrophage products such as H₂O₂, nitric oxide, and various cytokines might exert more noxious effects on hepatocytes (39). For example, TNF-α is known to induce UCP-2 gene expression in hepatocytes (4), an effect that might limit the efficiency of mitochondrial ATP production in these cells, predisposing them to necrosis (2). KC-derived cytokines, such as IL-15, also play a key role in modulating the differentiation and proliferation of other cells that regulate innate immunity (18). The importance of IL-15 in the regulation of the innate immune system is well illustrated by studies of IL-15 transgenic mice and mice that are genetically deficient in the β-chain of IL-2/IL-15 receptor, which is required for IL-15 signaling. IL-15 prevents the apoptosis of natural killer (NK) cells and certain lymphocytes (38). Consequently, IL-15 transgenic mice accumulate massive numbers of NK cells and CD8 (+) T cells (8), whereas mice that lack IL-15 function are severely depleted of these types of cells (12). Other KC-derived cytokines, such as IL-12 and -18, also regulate NK cell differentiation, with either of these cytokines promoting the local expansion of cytotoxic NK cell subpopulations that express large amounts of IFN within the hepatic microenvironment (16). The expansion or depletion of various cellular components of the innate immune system has enormous implications, including dramatic changes in hepatic vulnerability to LPS. For example, agents such as Propionibacterium acnes that activate macrophage IL-12 production selectively reduce hepatic CD4(+)NK T cells and markedly potentiate subsequent LPS-induced liver injury (23). Hepatic CD4(+)NK T cells are significantly reduced in ob/ob mice (9), which also exhibit enhanced vulnerability to LPS hepatotoxicity (6, 40). The mechanism for hepatic CD4(+)NK T cell depletion in ob/ob mice is unknown, but several potential causes have been identified (A. M. Diehl, S. Q. Yang, H. Z. Lin, R. Schwenk, and U. Kryzch, unpublished observations), including increased KC production of IL-12 and decreased KC production of IL-15. Overnight incubation of ob/ob KC in leptin-containing medium fails to correct either abnormality. However, hepatic expression of IL-15 increases in ob/ob mice that have been treated with continuous subcutaneous infusion of leptin for 2 wk, and this is associated with partial repletion in hepatic CD4(+) NK T cells. In vivo leptin therapy also dramatically increases the hepatic expression of IL-10, an IL-15 inducible cytokine (1), following LPS exposure. This improved IL-10 response to LPS challenge might be part of the mechanism by which supplemental leptin protects ob/ob mice from LPS hepatotoxicity (6).

Given the previous discussion about the enhanced vulnerability of ob/ob livers to LPS-induced toxicity, it is surprising that ob/ob mice are unusually resistant to concanavalin A (ConA)-induced hepatitis (7). This is particularly puzzling because, similar to LPS-induced liver injury, ConA hepatitis is also mediated by TNF-α (36). However, unlike LPS, which primarily activates innate immune responses, ConA mediates hepatotoxicity by activating T cells (7, 26). Leptin is important for T cell expansion (32). Hence, leptin-deficient ob/ob mice have reduced numbers of thymocytes and peripheral blood CD4(+) T cells (11, 20). CD4(+) T cells are also reduced in the livers of ob/ob mice (9). Thus it is likely that reduced CD4(+) T cell populations help to protect ob/ob mice from ConA hepatitis. Overproduction of certain KC-derived cytokines such as IL-6 might also contribute to this phenotype, because IL-6 is known to protect normal mice from ConA hepatitis (26).

In summary, a number of abnormalities, including altered production of various cytokines, prostanoids, and reactive oxygen intermediates, has been identified in hepatic, peritoneal, and/or bone marrow macrophages from fa/fa rats and ob/ob mice, two animal models of NAFLD. The KC dysfunction in these rodents is likely to contribute to some of the mechanisms, such as the induction of certain mitochondrial proteins, including bcl-2-family members and UCP-2, that influence hepatocyte viability and energy homestasis. Altered KC production of various cytokines is also likely to deplete the liver of certain populations of lymphocytes, thereby changing vulnerability to the hepatotoxic actions of agents that activate both innate and T cell-mediated immune responses. Because the actions of leptin, an acknowledged immunomodulator, are reduced in leptin-resistant fa/fa rats and leptin-deficient ob/ob mice, it is uncertain to what extent, if any, these
findings can be extrapolated to humans or other animals with NAFLD. However, these results provide a framework on which a more extensive evaluation of KC function can be built.

REFERENCES


