The proinflammatory phenotype of PECAM-1-deficient mice results in atherogenic diet-induced steatohepatitis

Reema Goel, Brian Boylan, Lynn Gruman, Peter J. Newman, Paula E. North, and Debra K. Newman

1Blood Research Institute, BloodCenter of Wisconsin, Milwaukee, Wisconsin; 2The Department of Pathology and Laboratory Medicine, Children’s Hospital of Wisconsin, Milwaukee, Wisconsin; Departments of 3Pathology, 4Cell Biology, Neurobiology and Anatomy, 5Pharmacology, and 6Microbiology and Molecular Genetics, and 7The Cardiovascular Center, Medical College of Wisconsin, Milwaukee, Wisconsin

Submitted 11 April 2007; accepted in final form 2 October 2007

The proinflammatory phenotype of PECAM-1-deficient mice results in atherogenic diet-induced steatohepatitis. Am J Physiol Gastrointest Liver Physiol 293: G1205–G1214, 2007. First published October 11, 2007; doi:10.1152/ajpgi.00157.2007.—The severity of nonalcoholic steatohepatitis (NASH) is determined by environmental and genetic factors, the latter of which are incompletely characterized. Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a 130-kDa transmembrane glycoprotein expressed on blood and vascular cells. In the present study, we provide data for the novel finding that genetic deficiency of PECAM-1 potentiates the development and progression of NASH. We found that the rate of development and severity of diet-induced NASH are markedly enhanced in PECAM-1-deficient [knockout (KO)] mice relative to wild-type (WT) mice, as measured by histological and biochemical evaluation. Livers from KO mice exhibited typical histological features of NASH, including macrovesicular fat accumulation, hepatocyte injury with infiltration of inflammatory cells, fibrosis, and heightened oxidative stress. Alanine aminotransferase, a marker for liver injury, was also significantly higher in KO compared with WT mice. Consistent with a role for PECAM-1 as a suppressor of proinflammatory cytokines, plasma levels of inflammatory cytokines, including TNF-α and monococyte chemoattractant protein-1 (MCP-1), were also significantly higher in KO compared with WT mice. These findings are the first to show that the PECAM-1-deficient mouse develops progressive nonalcoholic fatty liver disease (NAFLD), supporting a role for PECAM-1 as a negative regulator of NAFLD progression. Future examination of recently identified PECAM-1 allelic isoforms in humans as potential risk factors for developing NASH may be warranted.

CD31; liver; NASH; inflammation

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) encompasses an array of liver pathologies observed in individuals who do not abuse alcohol. The disease spectrum ranges from accumulation of fat in hepatocytes (steatosis) to the presence of an inflammatory infiltrate and fibrosis [nonalcoholic steatohepatitis (NASH)], and ultimately to progressive fibrosis and cirrhosis (14, 42–44, 46). The steatosis that characterizes NAFLD is frequently associated with features of the metabolic syndrome, including intra-abdominal obesity, dyslipidemia, insulin resistance, type 2 diabetes, and hypertension (14, 44), which has led to the suggestion that NAFLD is the hepatic manifestation of the metabolic syndrome (3, 12). Because the metabolic syndrome is found with increasing frequency in association with a Westernized lifestyle, NAFLD is increasingly recognized as a frequent cause of liver dysfunction in Western societies, and is estimated to occur with a prevalence of ~25% in Western countries, although only a subset of affected individuals develop the more advanced forms of the disease (14, 16, 42). A two-hit model has been proposed to explain NAFLD and NASH progression (18, 20). The first hit is steatosis, which results from disrupted synthesis, transport, and removal of long chain fatty acids and triglycerides and sensitizes the liver to the occurrence of a second hit (3, 44). The second hit induces hepatocyte injury and inflammation and is critically dependent on oxidative stress and production of proinflammatory cytokines (3, 16, 42). The nature and severity of first and second hits can be influenced by both environmental (diet, drugs) and genetic variables (18, 43).

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a 130-kDa glycoprotein that is expressed at the junctions of all vascular cells (47). To date, PECAM-1 engagement or expression has been shown in a number of experimental systems to function as an inhibitory receptor that limits agonist-induced activation of blood and vascular cells (47). To date, PECAM-1 engagement or expression has been shown to inhibit T cell (49), B cell (70), mast cell (72), and platelet (25, 52, 57) reactivity and to inhibit production of proinflammatory cytokines in vivo (9, 41, 63). PECAM-1 has also been implicated in maintaining vascular integrity in at least four different in vivo models of inflammation, including intradermal injection of histamine (29), autoimmune encephalomyelitis (29), collagen-induced arthritis (63, 71), and lipopolysaccharide-induced endotoxemia (9, 41).

Since PECAM-1 is thought to be a negative regulator of inflammatory responses, and since progression of NAFLD to NASH is associated with chronic inflammation, we sought to determine the effect of PECAM-1 deficiency on development of NASH. Using a high-fat diet-induced mouse model of NAFLD, we found that, whereas both PECAM-1-deficient and wild-type (WT) mice developed steatosis on the diet, only PECAM-1-deficient mice exhibited steatohepatitis with associated liver injury, inflammation, oxidative stress, and fibrosis. Our studies demonstrate that PECAM-1 deficiency places mice at risk for development of NASH and support
future examination of recently identified PECAM-1 allelic isoforms as potential risk factors for developing NASH in humans.

MATERIALS AND METHODS

Animals and diet. WT C57BL6 mice and PECAM-1-deficient (KO) mice (22) that had been backcrossed for >12 generations onto a C57BL6 background, which display no evidence of liver disease during their lifetime when fed a normal diet (R. Goel and D. Newman, unpublished observations), were maintained in a facility free of well-defined pathogens under the supervision of the Biological Resource Center at the Medical College of Wisconsin. All animal protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin.

Six- to 8-wk-old male and female KO and WT mice were placed on either a normal diet (ND) (mouse chow 5010; Purina, St. Louis, MO), containing 13.4% of calories derived from fat, or a high-fat diet (Adjusted Calories Western Type Diet TD.05248; Harlan Teklad, Madison, WI), containing 42% of calories derived from fat and 0.5% sodium cholate, hereafter referred to as the atherogenic diet (AD). The mice were housed in groups of four per cage, maintained under alternating 12-h light-dark cycles, and had free access to food and water.

Plasma and serum lipid, cytokine, and liver enzyme analyses. Blood was collected by cardiac puncture of anesthetized mice, plasma and serum were prepared, and aliquots were stored at −80°C until analyzed. Plasma levels of total cholesterol, HDL cholesterol, and triglycerides were measured by enzymatic colorimetric assay per manufacturer instructions in individual plasma samples (3–5 µl) from

Fig. 1. Effect of an atherogenic diet on plasma lipid levels. Nonfasting plasma levels of total triglycerides (A) and total cholesterol (B) were measured in wild-type (WT, open bars) and PECAM-1-deficient (KO, solid bars) mice fed a normal diet (ND) or an atherogenic diet (AD) for 9 or 18 wk. Results are expressed as mean plasma lipid levels ± SE measured in the number of mice indicated at the base of the relevant bar. Plasma levels of total cholesterol but not triglycerides were significantly increased in both KO and WT mice fed an AD for 9 or 18 wk and relative KO or WT mice, respectively, fed an ND (†††P < 0.001).

Fig. 2. Histological characterization of AD-induced hepatic steatosis and inflammation. Representative photomicrographs showing liver histology of WT (A, C, E, and G) and KO (B, D, F, and H) mice fed an ND (A and B) or an AD for 3 (C and D), 9 (E and F), or 18 (G and H) wk. Note that steatosis and inflammation occur earlier and are more severe in KO than in WT mice on the AD [hematoxylin and eosin (H&E) stain; original magnification: ×20].
5–8 mice in each group using commercially available reagents (Wako Diagnostics, Richmond, VA). Plasma levels of the proinflammatory cytokines IL-6, IL-10, monocyte chemoattractant protein-1 (MCP-1), IFN-γ, TNF-α, and IL-12p70 were measured using the mouse inflammation Cytometric Bead Array (BD Biosciences, San Jose, CA) according to the manufacturer’s protocol. Plasma levels of aspartate aminotransferase (AST) and lactic acid dehydrogenase (LDH), as well as direct and total serum bilirubin and alanine aminotransferase (ALT) levels, were determined by the clinical laboratory at Children’s Hospital of Wisconsin.

**Liver histology.** Livers were collected and immediately divided into three portions. One portion was fixed in 10% zinc formalin and embedded in paraffin, and a second portion was embedded in optimal cutting temperature (OCT) and snap-frozen; both of these portions were used for histology. The third portion was snap frozen and used for fluorescence microscopy studies (see below). Sections (4 μm in length) of paraffin-embedded tissue were deparaffinized and stained with hematoxylin and eosin (H&E), reticulin/nuclear fast red (Dako, Carpinteria, CA) or Masson’s trichrome (Richard Allan, Kalamazoo, MI). Five- to 10-μm sections of frozen tissue were stained with Oil Red O (Sigma, St. Louis, MO) for 15 min, washed, and counterstained with hematoxylin (Dako) for 45 s. Liver histology was semiquantitatively scored in a blinded manner as previously described (36). In each specimen, at least 50 random microscopic fields were examined under ×40 high-power (HP) magnification. Specifically, microvesicular and macrovesicular steatosis were each individually evaluated in H&E-stained sections and scored on a scale of 0–4 as follows: 0, no steatosis; 1, 5% steatosis; 2, 5–33% steatosis; 3, 33–66% steatosis; 4, >66% steatosis. Lobular and sinusoidal inflammation were each individually evaluated in H&E-stained sections and scored on a scale of 0–3 as follows: 0, no inflammatory foci; 1, 1 inflammatory focus/HP field; 2, 2–3 inflammatory foci/HP field; 3, >4 inflammatory foci/HP field. Fibrosis was evaluated in reticulin- and trichrome-stained sections and scored on a scale of 0–4 as follows: 0, no fibrosis; 1, mild fibrosis (excess connective tissue within the portal tracts, but no extension into the adjacent parenchyma); 2, moderate portal/periportal fibrosis (fibrous tissue occupies the portal tracts and periportal region partially or completely with connective tissue extending into the neighboring parenchyma); 3, bridging fibrosis; 4, cirrhosis. The average mean scores for microvesicular steatosis, ma-
crovesicular steatosis, sinusoidal inflammation, lobular inflammation, reticulin fiber staining, and trichrome staining were summed and divided by six to obtain a mean total histological score for steatohepatitis.

Fluorescence microscopy for assessment of superoxide production. The oxidation-dependent fluorescent dye dihydroethidium (DHE; Molecular Probes, Invitrogen Detection Technologies, Eugene, OR) was used to evaluate production of superoxide. DHE is freely permeable to cells and, in the presence of superoxide, is oxidized to 2-hydroxyethidium, which fluoresces at 585 nm. Unfixed frozen livers were cut into 5–10-μm thick sections, which were placed on glass slides. Sections were pretreated for 1 h with 150 μM Mn(III)tetrakis(4-benzoic acid)porphyrin (MnTBAP) chloride (Alexis Biochemicals, Lausen, Switzerland) to scavenge extracellular superoxide (17, 28). DHE (5 μM) was then applied to the surface of each tissue section, and the slides were incubated in a light-protected, humidified chamber at 37°C for 30 min. Images were obtained with a fluorescent microscope (Zeiss Axioskop MC100; Carl Zeiss, Thornwood, NY) equipped with a krypton-argon laser. Identical laser settings were used for acquisition of images from specimens that were processed and imaged in parallel. Fluorescence was detected with a 585-nm filter.

Macrophage recruitment assay. To obtain macrophages, mice were injected intraperitoneally with 1 ml of 3% aged thioglycollate (Sigma). After 5 days, peritoneal exudate was obtained from thioglycollate-treated mice by peritoneal lavage using ice-cold 30% sucrose in phosphate-buffered saline. For adoptive transfer experiments, macrophages were labeled with 5 μM 5-(and-6)-carboxyfluorescein diacetate(CFDA-SE; Invitrogen Detection Technologies) (5, 51) for 20 min, and the efficiency of CFDA-SE labeling was verified by immunofluorescence. Macrophages (3 × 10⁶) in 200 μl of PBS were injected retro-orbitally into mice that had received the atherogenic diet for 8 wk. After 48 h, the recipient mice were killed, their livers, spleens, and lungs were isolated and frozen in OCT. Serial tissue cryosections were stained with 4′,6-diamidino-2-phenylindole (DAPI) and H&E and examined by light and fluorescence microscopy (Zeiss Axioskop MC100; Carl Zeiss) using identical laser settings.

Statistical analyses. Data are presented as means ± SE. Differences between means were analyzed by two-way ANOVA for PECAM-1 WT and KO mice over the time course of atherogenic diet using GraphPad Prism 4 software (GraphPad Software, San Diego, CA). When the interaction was significant (P < 0.05), the Bonferroni posthoc test was applied to test for differences between groups.

RESULTS

PECAM-1-deficient mice develop marked atherogenic diet-induced steatosis, inflammation, and fibrosis. The strong association between obesity, NAFLD, and NASH has prompted study of the effects of diet on the development of steatosis and steatohepatitis in mouse models (3, 37). The C57BL6 mouse is a particularly good model of the human metabolic syndrome because, like humans, C57BL6 mice develop obesity, hyperlipidemia, hyperinsulinemia, hypertension (30), and steatohepatitis (31, 65) when allowed ad libitum access to a high-fat atherogenic diet. To determine whether PECAM-1 deficiency affects the development of steatohepatitis in C57BL6 mice, 6- to 8-wk-old male and female KO and WT C57BL6 littermates were allowed ad libitum access to a normal chow diet or to a high-fat atherogenic diet (21.2% fat, 0.15% cholesterol, 0.5% sodium cholate) for up to 18 wk. PECAM-1 deficiency did not affect weight gain on the normal chow diet (data not shown) or on the atherogenic diet (supplemental Fig. 1; supplemental figures for this article are available online at the American Journal of Physiology Gastrointestinal and Liver Physiology website) at any time point on the diet, suggesting that dietary intake did not differ systematically between KO and WT mice. We also examined the effect of the atherogenic diet on lipid profile in these mice (Fig. 1). Whereas levels of total triglycerides did not increase at any time on the atherogenic diet in either WT or KO mice (Fig. 1A), total cholesterol levels were

![Fig. 4. Histological characterization of AD-induced lipid accumulation and fibrosis in the liver. Representative photomicrographs of liver sections from WT (A, C, and E) and KO (B, D, and F) mice fed an AD for 9 wk, after staining with Oil Red O as a measure of lipid accumulation (A and B), reticulin as a measure of fibrosis (C and D), and trichrome as a measure of collagen deposition (E and F) (original magnification: ×40).](http://ajpgi.physiology.org/)

**Fig. 4.** Histological characterization of AD-induced lipid accumulation and fibrosis in the liver. Representative photomicrographs of liver sections from WT (A, C, and E) and KO (B, D, and F) mice fed an AD for 9 wk, after staining with Oil Red O as a measure of lipid accumulation (A and B), reticulin as a measure of fibrosis (C and D), and trichrome as a measure of collagen deposition (E and F) (original magnification: ×40).
significantly higher \((P < 0.0001)\) in both WT and KO mice after 9 and 18 wk on the atherogenic diet relative to genetically identical littermates fed a normal diet. However, the higher levels of total cholesterol observed after 9–18 wk on the atherogenic diet did not differ significantly between WT and KO littermates (Fig. 1B). These results suggest that PECAM-1 deficiency does not affect the development of hypercholesterolemia on the atherogenic diet.

Histological analysis of H&E-stained liver sections revealed that after 3 wk on an atherogenic diet, WT and KO mice developed mild microvesicular steatosis (Fig. 2, C and D), predominantly in hepatic parenchymal cells in the centrilobular region. After 9 and 18 wk on the atherogenic diet, prominent steatosis and mild inflammation were seen in livers of WT mice (Figs. 2E and 3), whereas significantly more severe steatosis, lobular, and sinusoidal inflammation developed in the livers of KO mice (Figs. 2F and 3), consistent with the typical histological features of steatohepatitis. The presence of multinucleated hepatocytes and apoptotic cells was also observed. Histological features progressed to prominent steatosis and mild inflammation in WT mice (Figs. 2G and 3), whereas severe steatosis and conspicuous inflammation was evident in KO mice (Figs. 2H and 3) after 18 wk on the atherogenic diet. Bile duct hyperplasia was noted in some of the KO mice that were fed the atherogenic diet, but this did not correlate with increased bilirubin levels (data not shown).

Consistent with the histological analysis of H&E-stained liver sections, neutral lipid accumulation was markedly more pronounced in KO (Fig. 4B, supplemental Fig. 2) relative to WT (Fig. 4A) livers after 9 wk of atherogenic diet. Also, in contrast to WT mice, reticulin fiber (Fig. 4D vs. 4C, 3E, supplemental Fig. 3) and collagen deposition (Fig. 4F vs. 4E, 3F, supplemental Fig. 4) were markedly increased in the livers of KO mice after 9 and 18 wk on the atherogenic diet. The fibrosis was concentrated in the centrilobular area with no bridging between portal tracts, indicating focal fibrosis had developed by this time in KO but not WT mice. Our results indicate that PECAM-1 deficiency exacerbates the rate and extent of steatohepatitis in animals fed an atherogenic diet for at least an 18-wk time period. It remains possible that significant steatohepatitis eventually does develop in WT mice fed an atherogenic diet, but that requires a period of time longer than 18 wk.

**Fig. 5.** Effect of PECAM-1 deficiency on hepatocellular damage in response to ingestion of an AD. Plasma alanine aminotransferase (ALT) levels were determined in WT (open bars) and KO (solid bars) mice fed an ND or an AD for 9 or 18 wk. Results are expressed as mean ALT levels \(\pm SE\) with the number of mice in each group indicated at the base of each bar. Plasma ALT levels were significantly increased in KO relative to WT mice fed an AD for 9 wk (**\(P < 0.01\)*) and between KO mice fed an AD relative to KO mice fed an ND for 9 wk (†††\(P < 0.001\)).

Consistent with the histological analysis of H&E-stained liver sections, neutral lipid accumulation was markedly more pronounced in KO (Fig. 4B, supplemental Fig. 2) relative to WT (Fig. 4A) livers after 9 wk of atherogenic diet. Also, in contrast to WT mice, reticulin fiber (Fig. 4D vs. 4C, 3E, supplemental Fig. 3) and collagen deposition (Fig. 4F vs. 4E, 3F, supplemental Fig. 4) were markedly increased in the livers of KO mice after 9 and 18 wk on the atherogenic diet. The fibrosis was concentrated in the centrilobular area with no bridging between portal tracts, indicating focal fibrosis had developed by this time in KO but not WT mice. Our results indicate that PECAM-1 deficiency exacerbates the rate and extent of steatohepatitis in animals fed an atherogenic diet for at least an 18-wk time period. It remains possible that significant steatohepatitis eventually does develop in WT mice fed an atherogenic diet, but that requires a period of time longer than 18 wk.

**Fig. 6.** Histological characterization of hepatic oxidative stress. WT (A, C, E, and G) or KO (B, D, F, and H) mice fed an ND (A, B, E, and F) or an AD (C, D, G, and H) for 9 wk were stained with the superoxide-sensitive fluorescent dye, dihydroethidine, either alone (A–D) or following pretreatment with the superoxide scavenger MnTBAP (E–H). Note that superoxide levels were markedly higher in KO mice on an AD than in WT mice on an AD and in either KO or WT mice fed an ND.
PECAM-1-deficient mice have significant hepatocellular damage on an atherogenic diet. A number of blood tests are commonly used to assess hepatic damage and liver disease, including measurement of AST, ALT, LDH, alkaline phosphatase, and direct and total serum bilirubin, albumin, and prothrombin time (7). Studies show that in NASH plasma liver enzyme abnormalities are primarily restricted to elevations in ALT and/or AST (1, 23). Correspondingly, we observed no diet-induced changes in the levels of AST, direct bilirubin, or LDH in either WT or KO (data not shown). However, we found that plasma levels of ALT were significantly increased in KO mice after 9 wk on an atherogenic diet, relative to WT mice, and these levels return to normal after 18 wk as hepatocytes become depleted of enzyme content and are replaced by fibrotic tissue (21) (Fig. 5). These data indicate that the atherogenic diet induces more severe liver injury in PECAM-1-deficient mice than in WT mice.

PECAM-1-deficient mice exhibit pronounced hepatic oxidative stress and increased production of proinflammatory cytokines in response to an atherogenic diet. Oxidative stress and coincident or consequent production of proinflammatory cytokines play a central role in the pathogenesis of NASH and hepatic fibrosis (2, 3, 11, 20, 27, 43, 53). To understand the pathophysiological mechanisms responsible for the marked oxidative stress and proinflammatory cytokines in these animals. We probed for superoxide production as a marker of oxidative stress. As shown in Fig. 6, we found that levels of KO mice fed an atherogenic diet for 9 wk exhibited strikingly higher levels of superoxide anion compared with livers of WT mice, and the majority of superoxide in the livers could be scavenged by superoxide scavenger, MnTBAP. We next measured plasma levels of some of the proinflammatory cytokines and chemokines, including IL-6, IL-10, MCP-1, IFN-γ, TNF-α, IL-12p70 in WT and KO mice. We found no evidence for diet-induced changes in levels of IL-10, IFN-γ, or IL-12p70 in either WT or KO mice throughout the study (data not shown). However, in both WT and KO mice fed an atherogenic diet for 9 wk or 18 wk, plasma IL-6 levels were significantly increased relative to littermates fed a normal diet, with no significant differences between KO and WT mice (Fig. 7A). This finding is consistent with previous studies, which showed that the atherogenic diet, in general, leads to a state of chronic, subacute inflammation (4, 8, 19). Increases in plasma levels of MCP-1 (Fig. 7B) and TNF-α (Fig. 7C) were also evident in both WT and KO mice fed an atherogenic diet relative to genetically identical mice fed a normal diet for 18 wk; however, plasma levels of MCP-1 and TNF-α were significantly higher in KO relative to WT mice fed an atherogenic diet for 9 wk. Taken together, these results indicate that, upon ingestion of an atherogenic diet for 9 wk, hepatic oxidative stress and production of the proinflammatory cytokines MCP-1 and TNF-α are markedly exacerbated in KO mice.

Macrophage accumulation in the liver is enhanced in KO mice on an atherogenic diet. The activation and recruitment of leukocytes from blood into a site of inflammation is critical in the pathogenesis of liver diseases (34, 55). PECAM-1 homo-philic interactions have been reported to play an important role in leukocyte transendothelial migration (22, 59, 64); however, our finding that liver inflammation is increased in KO relative to WT mice is inconsistent with these previous findings. We injected hypercholesterolemic recipient mice with fluorescently labeled peritoneal macrophages from donor mice to directly determine leukocyte recruitment and the fate of recruited leukocytes in KO and WT mice. No noticeable difference in macrophage accumulation was evident in the spleens and lungs of WT relative to KO mice (data not shown). However, as shown in Fig. 8, marked macrophage accumulation in the liver was evident 48 h after injection when either WT or KO macrophages were introduced into KO mice relative to WT recipient mice, suggesting that leukocyte recruitment or retention into inflamed liver tissue is enhanced in the absence of PECAM-1.
DISCUSSION

The progression of NAFLD to NASH is thought to be determined by an interaction between genetic and environmental factors, both of which are difficult to control in human populations. Consequently, it is important to identify the genes and environmental variables that influence this disease process. In the present studies, we provide evidence that PECAM-1 deficiency, in combination with ingestion of an atherogenic diet, results in the development of pronounced steatohepatitis in mice. Specifically, histological evaluation of liver sections revealed development of severe microvesicular and macrovesicular steatosis, lobular and sinusoidal inflammation, and focal fibrosis; the rate and the extent of which were markedly enhanced in KO compared with WT mice fed an atherogenic diet and coincident with elevated plasma ALT levels, increased hepatic oxidative stress, and increased plasma levels of the proinflammatory cytokines TNF-α and MCP-1. PECAM-1 deficiency contributed to higher levels of oxidative stress and proinflammatory cytokine production, which have been identified as second hits capable of inducing positive feedback for further hepatocyte steatosis, along with hepatocyte injury and inflammation responsible for progression of simple steatosis to NASH (3, 12, 18, 20, 53). On the basis of our findings, therefore, we propose that loss of PECAM-1 from LSEC, Kupffer cells, and/or IHL results in production of high levels of ROS and inflammatory cytokines, which, in turn, profoundly affect, albeit indirectly, the function of hepatocytes.

The finding in the present studies that PECAM-1 deficiency is associated with heightened levels of oxidative stress and proinflammatory cytokine production is consistent with two sets of previous observations. First, we have previously reported that ROS, such as superoxide anion, hydrogen peroxide, and peroxynitrite, are overproduced in PECAM-1-deficient mice (39). Second, several recent studies have provided evidence that PECAM-1 suppresses production of proinflammatory cytokines. Specifically, plasma concentrations of IL-1, TNF-α, MCP-1, IFN-γ, and IL-6 were all found to be significantly increased 24 h following injection of lipopolysaccharide into PECAM-1-deficient relative to WT coronary microvessels (39). PECAM-1 is expressed on the surfaces of endothelial cells as well as most bone marrow-derived hematopoietic cells, including platelets, monocytes, granulocytes, and some lymphocytes (32). The liver is no exception to this paradigm, wherein PECAM-1 is expressed on liver sinusoidal endothelial cells (LSEC), Kupffer cells, and intrahepatic lymphocytes (IHL) but not on hepatocytes (45). The architectural organization of the liver allows for close proximity and even direct interaction between these cell groups (67), making it possible for soluble mediators produced by LSEC or leukocytes to affect nearby hepatocytes. Previous studies have shown that excessive exposure to either oxidative stress or inflammatory cytokines can induce hepatocyte apoptosis and dysfunction, which can exacerbate acute and chronic liver injury (15, 73). Of particular relevance to the present study, it has been speculated that reactive oxygen species (ROS) and proinflammatory cytokines are among the “second hits” that cause simple steatosis in the liver to progress to NASH, and ultimately, to fibrotic disease (3, 12, 18, 20, 53).

Fig. 8. Distribution of fluorescently labeled macrophages in mice fed the AD. Representative overlay photomicrographs of 4′,6-diamidino-2-phenylindole (DAPI) (white staining) and 5-(and-6)-carboxyfluorescein diacetate (CFDA-SE) (green staining) in liver sections from WT (A and B) or KO (C and D) mice fed an AD for 9 wk and injected with WT (A and C) or KO (B and D) macrophages for 48 h. Note that livers from KO mice show abundant infiltrated WT and KO macrophages compared with WT mice injected with either WT or KO macrophages.
diet remains to be determined. Likewise, the source of over-produced ROS in PECAM-1-deficient mice and the mechanism by which PECAM-1 suppresses ROS production remain fruitful areas of future research.

The recruitment of activated inflammatory cells into the liver is crucial for the pathogenesis of liver disease, and cell adhesion molecules play an important role in this process (34). PECAM-1 homophilic interactions have been reported to be important for leukocyte transendothelial migration (22, 59, 64). Nevertheless, we demonstrate in the present study that macrophages readily home into the inflamed livers of PECAM-1-deficient mice fed an atherogenic diet, which indicates that PECAM-1 homophilic interactions are not required for macrophage recruitment into the liver under these conditions. Our observation is consistent with the previous finding that PECAM-1 does not play a major role in transmigration of neutrophils into the liver during endotoxemia (13) and with the finding, in at least four different in vivo models of inflammation, that the blood vessels of PECAM-1-deficient mice are particularly susceptible to vascular leakage (9, 29, 41, 63, 71). Together, these results suggest either that PECAM-1 homophilic interactions are not as important for leukocyte transmigration into the liver as they are in other tissues or that increased vascular permeability in PECAM-1-deficient vessels overcomes the need for PECAM-1 homophilic interactions in transmigration of inflammatory cells into damaged tissue. The extent to which either mechanism contributes to the enhanced severity of NALFD observed in PECAM-1-deficient mice fed an atherogenic diet remains to be determined.

The NALFD phenotype is broad, affecting males and females, children and adults, and different ethnic populations, with widely variable outcomes ranging from a benign nonprogressive course to cirrhosis and liver failure (43, 44). Familial clustering of NASH and cirrhosis support a role for genetic polymorphisms in factors that predispose to NASH (43, 44, 46). PECAM-1 deficiency has thus far not been described in humans; however, the human PECAM-1 gene is polymorphic. A number of single nucleotide polymorphisms (SNPs) in the human PECAM-1 gene sequence have been reported (48, 60–62). Certain of these SNPs exist in linkage disequilibrium, resulting in the existence of at least four alleles within the human population (50). Although studies have not yet addressed the extent to which PECAM-1 alleles are associated with human disease, several human PECAM-1 SNPs have been found to correlate with progression of coronary artery disease (24, 26, 68, 69) and/or myocardial infarction (24, 38, 58). These findings suggest that PECAM-1 plays a role in the development of cardiovascular disease in humans. Since recent studies have led to the conclusion that the factors that predispose for development of cardiovascular disease are also risk factors for development of NASH (6, 11, 35, 40, 54, 56, 66), it will be important in future studies to determine whether any of the PECAM-1 SNPs or alleles are associated with an increased propensity to develop severe NALFD.

In summary, we demonstrate in the present study that PECAM-1 deficiency in mice fed an atherogenic diet contributes to development of more advanced stages of inflammatory liver disease. Whether PECAM-1 normally inhibits progression of NALFD by suppressing oxidative stress and inflammation or by maintaining an intact vascular permeability barrier are interesting and important questions that our present data cannot distinguish between and that remain to be addressed. It is also important to keep in mind that these mechanisms are not mutually exclusive. Nevertheless, our study identifies PECAM-1 as a potentially attractive target to elucidate the mechanisms leading to fatty liver disease pathogenesis and progression.

ACKNOWLEDGMENTS

The authors thank Marjorie Kipp for help in maintaining the mouse colony.

GRANTS

This work was supported by HL-40926 (to P. J. Newman and D. K. Newman) from National Heart, Lung, and Blood Institute of the National Institutes of Health, and by a Postdoctoral Fellowship (to R. Goel) from the American Heart Association.

REFERENCES

21. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. Recommendations for use of


