Inflammation and oxidative stress are considered critical factors in the progression of nonalcoholic fatty liver disease. Myeloperoxidase (MPO) is an important neutrophil enzyme that can generate aggressive oxidants; therefore, we studied the association between MPO and nonalcoholic fatty liver disease. The distribution of inflammatory cells containing MPO in liver biopsies of 40 severely obese subjects with either nonalcoholic steatohepatitis (NASH) \( (n = 22) \) or simple steatosis \( (n = 18) \) was investigated by immunohistochemistry. MPO-derived oxidative protein modifications were identified by immunohistochemistry and correlated to hepatic gene expression of CXC chemokines and M1/M2 macrophage markers as determined by quantitative PCR. MPO plasma levels were determined by ELISA. The number of hepatic neutrophils and MPO-positive Kupffer cells was increased in NASH and was accompanied by accumulation of hypochlorite-modified and nitrated proteins, which can be generated by the MPO-H\(_2\)O\(_2\) system. Liver CXC chemokine expression was higher in patients with accumulation of MPO-mediated oxidation products and correlated with hepatic neutrophil sequestration. Plasma MPO levels were elevated in NASH patients. Interestingly, neutrophils frequently surrounded steatotic hepatocytes, resembling the crown-like structures found in obese adipose tissue. Furthermore, hepatic M2 macrophage marker gene expression was increased in NASH. Our data indicate that accumulation of MPO-mediated oxidation products, partly derived from Kupffer cell MPO, is associated with induction of CXC chemokines and hepatic neutrophil infiltration and may contribute to the development of NASH. (Am J Pathol 2009, 175:1473–1482; DOI: 10.2353/ajpath.2009.080999)

The obesity-associated infiltration of neutrophils and macrophages into liver and adipose tissue is increasingly appreciated to be central to the development of chronic diseases such as nonalcoholic fatty liver disease (NAFLD) and insulin resistance.\(^1\) In both liver and adipose tissue, increased levels of free fatty acids are thought to initiate the activation of inflammatory pathways that are responsible for attraction and activation of immune cells.\(^2,3\) Furthermore, recruitment of neutrophils to the liver can be mediated by secretion of the CXC chemokines interleukin (IL)–8 and growth-related oncogene (GRO)–\(\alpha\).\(^4,5\) The inflammatory processes in the liver have been shown to play a particularly important role in the progression of the benign steatotic stage of NAFLD to the more advanced stages of nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis.\(^6,7\)

Next to inflammation, oxidative stress has been put forward as an important contributor to the progression of NAFLD.\(^8,9\) For example, oxidative stress leading to lipid peroxidation induces the progression of steatosis toward necroinflammation or fibrosis in both animal models of steatohepatitis and humans with steatotic liver disorders.\(^10–13\) Oxidative stress in the fatty liver can result from cytochrome P450 activity, peroxisomal \(\beta\)-oxidation, mitochondrial electron leak, as well as activities of recruited inflammatory cells.\(^14\)

One of the principal molecules released after recruitment and activation of phagocytes is myeloperoxidase (MPO), an important enzyme involved in the generation of reactive
oxygen species.\textsuperscript{15} MPO is highly expressed by neutrophils and as such widely used as a neutrophil marker. In the presence of physiological chloride concentrations, MPO reacts with hydrogen peroxide (H$_2$O$_2$, formed by the respiratory burst) to catalyze formation of hypohalous acid/ hypochlorite (HOCl/OCl\textsuperscript{-}) and other oxidizing species.\textsuperscript{15} These oxidants may contribute to host tissue damage at sites of inflammation through reactions with a wide range of biological substrates, including DNA, lipids, and protein amino groups.\textsuperscript{16} In the absence of physiological chloride concentrations, the MPO-H$_2$O$_2$ system can also form reactive nitrogen species\textsuperscript{17} that may initiate lipid peroxidation or form protein tyrosine residues, another posttranslational modification found in many pathological conditions.\textsuperscript{18}

Besides its abundant presence in neutrophils, where MPO makes up \textasciitilde{}5% of the total cell protein content, lower levels of MPO have been found in monocytes, certain macrophages,\textsuperscript{19–21} and in a subpopulation of Kupffer cells.\textsuperscript{22} The presence of MPO in these cells may reflect their inflammatory status, which was recently shown to be modulated in obesity. For example, anti-inflammatory M2 macrophages with tissue-remodeling capacities were largely absent in obese adipose tissue in mice, whereas accumulation of proinflammatory M1 macrophages was associated with obesity and insulin resistance.\textsuperscript{23} Moreover, attenuation of the M2 differentiation program of Kupffer cells was associated with hepatic steatosis.\textsuperscript{24} Interestingly, M1 macrophages are known to generate high amounts of reactive oxygen and nitrogen species.\textsuperscript{25} Therefore, MPO-containing M1 macrophages/Kupffer cells could play a role in NAFLD.

To study the potential contribution of increased MPO activity to the progression of human NAFLD, we quantified inflammatory cells that express MPO and assessed hepatic accumulation of HOCl-modified proteins and nitrotyrosine. Furthermore, we determined hepatic gene expression of IL-8 and GRO-\textalpha in relation to MPO-derived tissue damage and hepatic neutrophil sequestration to investigate a potential link between MPO activity and neutrophil accumulation. In addition, we analyzed for the first time hepatic gene expression of M1 and M2 macrophage phenotype markers in human NAFLD. Our results support an important role for Kupffer cell MPO-induced oxidative stress in the progression of NAFLD.

### Materials and Methods

#### Liver Specimens

Human liver specimens were obtained during bariatric surgery from 40 severely obese patients (body mass index \textasciitilde{} 40). None of the patients had suffered from viral hepatitis or autoimmune-related disorders or reported excessive alcohol consumption (\textasciitilde{}20 g/day). Each liver specimen was divided into three pieces. One part was used for RNA isolation, another part was fixed in formalin, and a third part was embedded in Tissue-Tek optimal cutting temperature compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) and snap-frozen in 2-methylbutane. Biopsies were stained with H&E, periodic acid-Schiff with dia-
nous peroxidase activity was blocked with 0.3% H2O2 in at 20°C with acetone, dried, and rehydrated. Endogenous peroxidase activity was blocked with 0.3% H2O2 in methanol, and nonspecific binding sites were blocked with goat serum in PBS. Sections were incubated with rabbit-anti-MPO/mouse-anti-HNP1-3 or rabbit-anti-MPO/mouse-anti-EMR1, rinsed with PBS, and subsequently incubated with Alexa Fluor 594-conjugated goat anti-rabbit IgG (1:50; Jackson ImmunoResearch). Nuclei were stained with 4′,6-diamino-2-phenylindol, and sections were mounted with Fluorescent Mounting Medium (Dako).

Quantification of Immunohistochemical Stainings

For the HNP1-3, MPO, and CD68 stainings, four to six randomly selected different parts of stained sections were photographed using a Zeiss Axioscope (Zeiss, Göttingen, Germany) and a standard charge-coupled device camera (Stemmer Imaging, Puchheim, Germany) at x200 magnification. Pictures were analyzed with Leica QWin image analysis software (Leica Microsystems, Cambridge, UK). Positive cell number was recorded as well as the extent of staining, and mean steatotic hepatocyte size was calculated by dividing the steatotic area by the number of hepatocytes showing steatosis. For the staining of HOCl-modified and nitrotyrosine epitopes, whole sections were observed and classified according to presence or absence.

MPO ELISA

Blood samples were collected the day before surgery after at least 8 hours of fasting using evacuated tubes containing EDTA and processed as described previously. Plasma MPO concentrations were measured using a sandwich ELISA for human MPO according to the manufacturer’s protocol (HyCult Biotechnology). All plasma samples were analyzed in duplicate in the same run. The intra-assay coefficient of variance was <10%.

Quantitative Real-Time PCR

Total RNA was isolated from 50 mg liver tissue by homogenization in Tri-reagent (Sigma-Aldrich), according to the manufacturer’s instructions. RNA (750 ng) was converted to cDNA using the iScript cDNA synthesis kit (ABgene, Leusden, The Netherlands) and the iQ5 iCycler (Bio-Rad, Hercules, CA). Quantitative real-time PCR was performed with the ABSolute SYBR Green Master Mix (ABGene, Leusden, The Netherlands) and the IQ5 iCycler (Bio-Rad). Relative expression was assessed in duplicate by the delta cycle threshold method after normalization for β-actin and β2-microglobulin expression. Sequences of the applied PCR primers are listed in Table 2.

Statistical Analysis

Results are presented as mean ± SEM. Statistical analyses were conducted using SPSS software (version 15.0). The Mann-Whitney rank sum test, the Kruskal-Wallis one-way analysis of variance, and the χ2 analysis were applied to study differences among the study groups, and associations between parameters were determined through Spearman correlation analysis. Values of P < 0.05 were considered statistically significant.

Results

Increased MPO-Positive Cell Number in NASH Is Associated with Extent of Steatosis

Forty-five percent (n = 18) of the 40 patients that were included in our study showed simple hepatic steatosis without significant inflammation, whereas 55% (n = 22) were diagnosed with NASH. Characteristics of the study groups with respect to the degree of steatosis, inflammation, and fibrosis are shown in Table 3. In comparison with the simple steatosis group, patients with NASH showed a significantly higher degree of steatosis as determined semiquantitatively by the pathologist (P < 0.001). Quantitative

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRO-α</td>
<td>5′-CCACTGGCGCCAAAAC-3′</td>
<td>5′-GCAGGATTGAGCAGCTT-3′</td>
</tr>
<tr>
<td>IL-8</td>
<td>5′-CTGGGTCGCTGCGAG-3′</td>
<td>5′-TTAGGCGCTATGCTCAA-3′</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5′-CTAGACCAAGCTTACCCG-3′</td>
<td>5′-GGCAAGCAGAGGAGA-3′</td>
</tr>
<tr>
<td>IL-10</td>
<td>5′-GAGGCTGCTACGATGTT-3′</td>
<td>5′-GGGACCTTACAGTGGCTAA-3′</td>
</tr>
<tr>
<td>Dectin-1</td>
<td>5′-CTAGGACACAAAGCTTACCCG-3′</td>
<td>5′-GAAGAGCTCTGGAGAGAC-3′</td>
</tr>
<tr>
<td>EMR-1</td>
<td>5′-GCTGTCGCTACGATGCTTA-3′</td>
<td>5′-CGCAGCAGCAGTACCTCT-3′</td>
</tr>
<tr>
<td>β-actin</td>
<td>5′-TCATCCCAGCAATGATGGT-3′</td>
<td>5′-GCATGACACTGGCATCTTTTG-3′</td>
</tr>
<tr>
<td>β2-microglobulin</td>
<td>5′-CCATCCCAGCAATGATGGT-3′</td>
<td>5′-CCATCCCAGCAATGATGGT-3′</td>
</tr>
</tbody>
</table>

EMR-1, epidermal growth-factor-like module containing mucin-like hormone receptor 1.
analyses also revealed that there was significantly more steatosis in patients with NASH as compared with patients with simple steatosis (**P < 0.001) (Figure 1A). In addition, the mean size of steatotic hepatocytes was significantly higher in the NASH group (**P < 0.001) (Figure 1A).

To further characterize the cells contributing to inflammation, we first identified neutrophils by immunohistochemical detection of the specific marker HNP1-3 (also known as α-defensin30) and by detection of MPO. Interestingly, HNP1-3-positive and MPO-positive neutrophils were frequently associated with lipid-laden hepatocytes (Figure 1B), forming structures that strikingly resemble the so-called “crown-like structures” commonly found around necrotic adipocytes in obese adipose tissue.

The degree of neutrophil infiltration into the liver varied considerably among the patients. As expected, the number of HNP1-3-positive neutrophils as well as the number of MPO-positive cells were significantly higher in the NASH group compared with the steatosis group (Figure 1C). In addition, the number of MPO-positive cells was higher in patients with grade 2 or 3 portal inflammation as compared with patients showing grade 1 (**P < 0.001; Figure 1D). Higher degrees of lobular inflammation were also associated with more MPO-positive cells (**P < 0.05; Figure 1D). Furthermore, the number of MPO-positive cells correlated with both the extent of steatosis (rs = 0.57, **P < 0.001) and mean steatotic hepatocyte size (rs = 0.52, **P < 0.001). In line with this observation, the number of MPO-positive cells was significantly higher in patients with a higher grade of steatosis as scored by the pathologist (**P = 0.03) (Figure 1E). On the other hand, the number of MPO-positive cells was un-

Table 3. Semiquantitative Analysis of the Degree of Steatosis, Inflammation, and Fibrosis in the Study Groups according to the Brunt Classification

<table>
<thead>
<tr>
<th></th>
<th>Steatosis</th>
<th>NASH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 (&lt;33% of hepatocytes)</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Grade 2 (33–66% of hepatocytes)</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Grade 3 (&gt;66% of hepatocytes)</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Portal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Stage 3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

χ² analysis for steatosis: **P < 0.001.
related to the degree of fibrosis ($P = 0.75$) (Figure 1F). Similarly, whereas the number of HNP1-3-positive cells was somewhat increased in the group of patients with stage 1 fibrosis ($P = 0.05$), patients with stage 2 or 3 fibrosis displayed a comparable number of HNP1-3-positive cells as patients without fibrosis (stage 0) (Figure 1F). Activation of hepatic stellate cells as reflected by immunohistochemical detection of α-smooth muscle actin was not associated with steatotic hepatocytes or NAFLD severity (data not shown).

**Increased Prevalence of MPO-Positive Kupffer Cells in NASH**

Interestingly, there were more MPO-positive cells than HNP1-3-positive cells, particularly in the NASH livers (Figure 1C). However, all HNP1-3-positive cells showed positivity for MPO (Figure 2A). This suggested that MPO-expressing cells other than neutrophils, most likely resident or infiltrating macrophages, were increased in NASH. Indeed, close examination of MPO-positive cells revealed that many of them lacked the multilobular nucleus characteristic of neutrophils. To further investigate the nature of these cells, double stainings using anti-MPO and anti-CD68 antibodies were performed. MPO/CD68-double-positive cells with a Kupffer cell-like morphology were frequently detected in patients with NASH (Figure 2B). Because the number of CD68-positive cells did not differ between the NASH group and patients with simple steatosis ($P = 0.28$; Figure 2C), this indicates that there is a selective increase in the population of MPO-expressing Kupffer cells in patients with NASH. Of note, many of these MPO-positive Kupffer cells were observed close to crown-like structures around steatotic hepatocytes (Figure 2B).

**Hepatic Accumulation of HOCl-Modified and Nitrated Proteins in NASH**

We next investigated whether the increased staining for immunoreactive MPO in the liver of NASH patients trans-
lated into accumulation of MPO-derived oxidative modifications. HOCl-modified epitopes were detected in 77% ($n = 17$) of the subjects in the NASH group. In contrast, only 22% ($n = 4$) of patients with simple steatosis showed staining for HOCl-modified epitopes. The extent and intensity of the HOCl-staining varied between individuals (Figure 3A). In some cases, more pronounced staining was associated with steatotic hepatocytes. Omission of the primary antibody 2D10G9 or preabsorption with HOCl-modified (lipo)protein prevented antibody binding (data not shown), confirming that the staining was specific for HOCl-modified epitopes generated by the MPO-H$_2$O$_2$-chloride system. Importantly, the number of hepatic MPO-positive cells was significantly higher in patients that show HOCl-modified protein staining ($P = 0.02$). In contrast, HNP1-3-positive cell number is not significantly increased in this group ($P = 0.14$; Figure 3B). Representative immunostainings of nitrotyrosine residues (red/brown) in livers of two patients with NASH (magnification, $\times 200$). More intense staining is predominantly associated with steatotic hepatocytes. As expected, the vast majority of patients showing staining for HOCl-modified proteins also showed pronounced staining for nitrosylated proteins (Figure 3D).

Increased Plasma MPO Levels in NASH Patients

Previously, we found elevated plasma MPO levels in severely obese patients compared with normal weight controls. On the basis of the remarkable increase of both MPO-positive cells and MPO-derived staining for HOCl-modified and nitrate-released epitopes in the liver of NASH patients, we hypothesized that plasma MPO levels could be higher in the subgroup of severely obese patients with NASH. Indeed, plasma MPO levels were significantly elevated in the NASH group compared with the patients with simple steatosis (59.5 ± 6.5 versus 41.1 ± 4.8 ng/ml, $P = 0.03$; Figure 4). However, there was no significant
correlation between plasma MPO levels and the number of MPO-positive cells in the liver.

Expression of CXC Chemokines Correlates with Neutrophil Accumulation in NAFLD

Recruitment of neutrophils to inflammatory sites is potentially induced by secretion of the CXC chemokines IL-8 and GRO-α, a process that occurs in response to cellular injury such as accumulation of chlorinated or nitrated macromolecules. Therefore, we investigated the expression of these chemokines in the liver of severely obese patients and studied their relation with MPO-derived oxidative modifications and neutrophil sequestration. Expression of both CXC chemokines was higher in patients that displayed simultaneous accumulation of HOCl-modified and nitrotyrosine epitopes when compared with patients without such accumulation (P = 0.05 and P = 0.04, respectively; Figure 5A). Moreover, expression of both GRO-α and IL-8 correlated with the number of HNP1-3-positive neutrophils (GRO-α, r_s = 0.42, P < 0.01; IL-8, r_s = 0.44, P < 0.01; Figure 5B). In line with our observation that many MPO-positive cells in the liver of NASH patients are not necessarily neutrophils, expression of these CXC chemokines did not correlate with the number of MPO-positive cells. As expected, expression of IL-8 and GRO-α was higher in the NASH group, although the difference with the simple steatosis group was not statistically significant (P = 0.76 and P = 0.64, respectively; Figure 5C).

M2 Macrophage Phenotype Markers Show Higher Hepatic Expression in NASH

The increased immunohistochemical staining for MPO in Kupffer cells in patients with NASH suggested that these cells might have a proinflammatory M1-like phenotype. To investigate this hypothesis, we performed mRNA expression analysis of several macrophage phenotype markers. Expression of IL-6, which is associated with the M1 phenotype, was markedly elevated in the NASH group compared with the simple steatosis group (Figure 6A), whereas expression of IL-1β, another M1 marker, was not altered (Figure 6A). This finding may suggest that the proinflammatory M1 phenotype is not dominant in NASH. In line with this, the expression of dectin-1, epidermal growth factor-like module containing mucin-like hormone receptor 1, and IL-10, all markers of anti-inflammatory M2 macrophages, was significantly higher in the liver of NASH patients (Figure 6B).

Discussion

Inflammation and oxidative stress are thought to be key elements in the progression of NAFLD. We have studied the potential contribution of MPO, an enzyme associated with oxygen/nitrogen species generation and inflammation, to the progression of human NAFLD. The data obtained in this study indicate that there are increased numbers of MPO-positive Kupffer cells in NASH. Furthermore, we show that accumulation of HOCl-modified and nitrated proteins is associated with CXC chemokine expression and infiltration of neutrophils into the liver. The potential role of MPO in NASH is further underscored by the fact that plasma MPO levels were elevated in NASH patients compared with patients with similar body mass index without NASH. Taken together, these results provide new evidence that increased expression and activity of MPO are associated with the perpetuating chronic inflammation that is observed in NASH.

MPO-positive neutrophils have previously been shown to be associated with the presence of oxidized phospholipids, in particular phosphatidylcholine, in steatotic hepatocytes. Importantly, oxidized phosphatidylcho-
line was also detected in Kupffer cells and shown to be more abundant in NASH than in normal livers. Our findings suggest that increased Kupffer cell MPO activity represents an additional source of oxidative damage in NASH. Also, intracellular MPO-mediated lipid peroxidation processes could represent an alternative route for formation of oxidized phospholipids in addition to the scavenger-receptor-mediated pathways that were previously proposed to be responsible.10

Although we have no direct evidence that MPO is involved in protein nitration in NASH, relevant nitration mechanisms via MPO are known to exist.18 The oxidation of nitrite by HOCl generated by the MPO-H2O2-halide system represents one route for nitrosyrosine formation.33,34 Alternatively, the MPO-H2O2 system may use nitrite to generate reactive nitrogen intermediates that promote nitration of proteins and lipid peroxidation.17 Most importantly, lysine-derived chloramines, formed by reaction of HOCl with protein amines, can generate nitrogen-centered radicals; these radicals are also formed by HOCl-derived modification of phospholipids.26 Interestingly, staining for HOCl-modified epitopes was frequently paralleled by intense staining for nitrosyrosine in our study. Comparable data have been obtained in diseased kidney tissue where lipid peroxidation and HOCl-modified proteins may occur at similar locations.37

Fragmentation of the extracellular matrix by MPO-derived HOCl has previously been shown to cause activation of hepatic stellate cells, thereby promoting hepatic fibrosis.40 Moreover, lipid peroxidation via tyrosyl radicals and nitrogen dioxide radicals (both formed by the MPO-H2O2 system) increases extracellular matrix synthesis by hepatic stellate cells as well.41 However, the number of MPO-positive cells was not associated with the stage of fibrosis in the present study, suggesting that MPO-derived products play a minor role in the activation of stellate cells in NASH in younger patients. Nevertheless, increased MPO activity in the long run may contribute to the progression of fibrosis.

Like other macrophages, Kupffer cells can be differentially activated according to the inflammatory microenvironment, leading to different biological properties.42 The increased presence of MPO in Kupffer cells in NASH as observed in this study raised the intriguing possibility that Kupffer cells gain M1 macrophage-like proinflammatory properties in NASH, because this type of macrophage is known to generate high amounts of reactive oxygen species.25 The increased expression of the established M1 macrophage marker IL-6 was consistent with this concept. However, expression of IL-1β, another prototypical M1 marker, was not elevated. Moreover, all M2 markers measured in the present study showed substantial up-regulation in NASH, suggesting that part of the inflammatory response of Kupffer cells in NASH is directed toward tissue remodeling. Of note, M2 macrophages produce transforming growth factor-β and other proteins involved in tissue remodeling, such as metalloproteinases 1 and 12.43 As such, these cells may contribute to fibrosis in NASH. In addition, M2 macrophages express many types of scavenger receptors implicated in the uptake of lipids, lipoproteins, and apoptotic cells. Taken together, these characteristics suggest a role for Kupffer cells in liver remodeling and tissue repair after hepatocyte necrosis because of excessive lipid accumulation, although we cannot rule out that hepatocytes other than Kupffer cells contribute to the elevated M2 marker gene expression.

In the present study, we found that hepatic expression of IL-8 and GRO-α was elevated in patients with oxidative hepatocyte damage. Moreover, their expression significantly correlated with neutrophil sequestration. This suggests that these CXC chemokines provide a molecular link between oxidative hepatocyte injury and the recruitment of neutrophils to the liver in NASH. Furthermore, it was recently reported44 that accumulation of triglycerides in hepatocytes causes a dose-dependent increase in IL-8 secretion. Thus, triglyceride-induced IL-8 secretion may be a factor that directly links steatosis and infiltration of the liver by neutrophils. Interestingly, HOCl-modified (lipo)protein has also been reported to promote IL-8 expression in human monocytes.45 Moreover, IL-8 plasma levels are elevated in human NASH.46 Our results suggest that secretion of GRO-α might also be of interest in this respect, and putative effects of triglyceride accumulation on GRO-α secretion by hepatocytes merit further study.

The frequent organization of neutrophils in crown-like structures around steatotic hepatocytes that we observed might be significant because the majority of macrophages in obese adipose tissue are also organized into this type of structure, which is thought to prevent leakage of potentially cytotoxic lipid droplets into the interstitium.47,48 This suggests that excessive fat accumulation in adipose tissue and liver activates a common mechanism that drives infiltration and organization of inflammatory cells into crown-like structures. Because this inflammatory structure is associated with necrotic cell death in adipose tissue,47,48 necrosis may also be a major stimulus for liver

![Figure 6. Increased hepatic expression of M2 macrophage phenotype markers in NASH.](AJP October 2009, Vol. 175, No. 4)
inflammation in NASH. The emergence of a chronic inflammatory reaction in response to necrotically dying cells has recently been coupled to the release of so-called damage-associated molecular pattern molecules or alarmins, endogenous proteins that act extracellularly to induce inflammatory responses.  

Interestingly, it has been suggested that oxidative processes are involved in the eradication of damage-associated molecular patterns, thereby modulating the effects of chronic inflammation toward tissue remodeling and repair.  

It is tempting to speculate that MPO-mediated oxidation contributes to this process and complements the tissue repair that is exerted by Kupffer cells with M2 properties.

In conclusion, this is the first study to show that the inflammatory process in NASH is characterized by an increased prevalence of MPO-positive Kupffer cells accompanied by significant MPO-mediated oxidative hepatocyte damage. In view of the association of MPO-mediated hepatocyte damage with both CXC chemokine expression and hepatic neutrophil infiltration, increased MPO activity might be a driving factor underlying the progression of human NASH. MPO could therefore represent a novel therapeutic target in human NASH.

Acknowledgments

We thank Niels Mels for technical assistance and Froukje Verdam and Petra Niessen for critical evaluation of the manuscript.

References


