Role of Nrf2 in the Regulation of CD36 and Stress Protein Expression in Murine Macrophages

Activation by Oxidatively Modified LDL and 4-Hydroxynonenal

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Abstract—CD36 is an important scavenger receptor mediating uptake of oxidized low-density lipoproteins (oxLDLs) and plays a key role in foam cell formation and the pathogenesis of atherosclerosis. We report the first evidence that the transcription factor Nrf2 is expressed in vascular smooth muscle cells, and demonstrate that oxLDLs cause nuclear accumulation of Nrf2 in murine macrophages, resulting in the activation of genes encoding CD36 and the stress proteins A170, heme oxygenase-1 (HO-1), and peroxiredoxin 1 (Prx 1). 4-Hydroxy-2-nonenal (HNE), derived from lipid peroxidation, was one of the most effective activators of Nrf2. Using Nrf2-deficient macrophages, we established that Nrf2 partially regulates CD36 expression in response to oxLDLs, HNE, or the electrophilic agent diethylmaleate. In murine aortic smooth muscle cells, expressing negligible levels of CD36, both moderately and highly oxidized LDL caused only limited Nrf2 translocation and negligible increases in A170, HO-1, and Prx 1 protein levels. Because PPAR-γ can be activated by oxLDLs and controls expression of CD36 in macrophages, our results implicate Nrf2 as a second important transcription factor involved in the induction of the scavenger receptor CD36 and antioxidant stress genes in atherosclerosis. (Circ Res. 2004;94:●●●●●●.)

Key Words: CD36 ● Nrf2 ● oxidized LDL ● macrophages ● vascular smooth muscle cells

Low-density lipoprotein (LDL) is susceptible to oxidative damage, and oxidatively modified LDL (oxLDL) plays a key role in the development of atherosclerotic lesions. OxLDL is taken up via different scavenger receptors, and in macrophages, CD36, SR-A, and LOX-1 are the predominant receptors. Enhanced formation of oxLDL in the vascular wall induces the formation of foam cells that accumulate cholesterol. LDL oxidation is associated with the formation of a number of highly reactive molecules, such as lipid peroxides, lysophosphatidylcholine, oxysterols, and aldehydes, which cause vascular inflammation and fibrosis and expression of antiinflammatory genes in macrophages and vascular smooth muscle cells.

Components of oxLDL, such as 9-HODE and 13-HODE, activate peroxisome proliferator-activated receptor γ (PPAR-γ), resulting in the upregulation of the major oxLDL receptor CD36. PPAR-γ appears to inhibit inflammatory response genes by antagonizing the activities of AP-1, NF-κB, and Stat 1 transcription factors. Moreover, macrophages derived from mice in which the PPAR-γ gene has been “floxed out” fail to upregulate CD36 expression in response to treatment with thiazolidinedione drugs such as rosiglitazone.

Our previous studies in vivo established that the transcription factor Nrf2, which interacts with electrophile (EpRE) and antioxidant (ARE) response elements and regulates expression of the detoxifying enzymes GST and NQO1 in tissues in response to dietary 2(3)-butyl-4-hydroxyanisole. Moreover, the sensitivity to carcinogenesis is increased in Nrf2-deficient mice due to the loss of induction of ARE-regulated drug metabolizing enzymes and antioxidant genes, and Nrf2 has also been implicated in the protection against oxidative damage induced by hyperoxia. Thus, Nrf2 serves as a key transcription factor in the cytoprotection of tissues against electrophiles and reactive oxygen species.

We previously reported that Nrf2 regulates expression of HO-1, Prx 1, anionic amino acid transporter xCT, and the ubiquitin/PKC-ζ-interacting protein A170 in murine peritoneal macrophages. Activation of Nrf2 by electrophilic agents or reactive oxygen species is controlled by a novel cytoplasmic protein designated Keap 1 that interacts with Nrf2 and negatively regulates nuclear translocation of Nrf2 and facilitates degradation of Nrf2 via the proteasome.

OxLDLs upregulate HO-1 and Prx 1 expression in murine macrophages and porcine aortic smooth muscle cells.
whereas immunostaining and in situ hybridization have established that HO-1 is prominently expressed in the endothelium and foam cells/macrophages in the intima of atherosclerotic lesions in humans and mice.24 HO-1 plays an essential antiinflammatory role in vitro and in vivo.25 and Prx I due its thiolredoxin peroxidase activity can reduce hydrogen peroxide26 and modulate H₂O₂-mediated activation of NF-κB.27

We have investigated the role of Nrf2 in the induction of stress proteins by oxLDLs and 4-hydroxynonenal (HNE) in murine peritoneal macrophages and aortic smooth muscle cells (SMCs) isolated from wild-type and nrf2-knockout mice. We show for the first time that oxLDLs activate Nrf2 in murine macrophages, but less efficiently in SMCs, and that HNE, one of the major end products of lipid oxidation and contained in oxLDL, is a potent activator of Nrf2 translocation to the nucleus in both cell types. Moreover, we provide novel evidence that Nrf2 is an important regulator of CD36 expression in murine macrophages. Because PPAR-γ can be activated by oxLDLs and controls expression of CD36 in macrophages, our results implicate Nrf2 as an additional transcription factor involved in the regulation of oxLDL uptake by the vascular wall and induction of antioxidant stress genes in atherosclerosis.

Materials and Methods

Reagents
4-Hydroxy-2-nonenal (HNE) and 15-deoxy-Δ12,14-PGJ₂ (15d-PGJ₂) were from Calbiochem, roseigalactone was from Cayman Chemical, HPODE was from Biomol, monolein antioxidant reactive with murine CD36 was from Cascade Biosciences, and Dr. Western (marker for Western blot) was from Oriental Yeast Co. Ltd. All other chemicals, actin antibody (A-2066), lysophosphatidylcholine (LPC), 7-ketocholesterol, hexanal, malondialdehyde, and 7β-hydroxycholesterol were from Sigma Chemical Co.

Culture of Mouse Peritoneal Macrophages and Aortic Smooth Muscle Cells

Mouse peritoneal macrophages were prepared from female ICR mice and nrf2-knockout mice backcrossed with ICR mice that 4 days previously received an intraperitoneal injection of 2 mL of 4% thioglycollate broth.20 Additional experiments were performed using peritoneal macrophages from CD36-deficient female mice. Macrophages were maintained in RPMI 1640 medium (10% fetal calf serum), chemical agents added and cultured as indicated. Explant cultures of mouse aortic smooth muscle cells (SMCs) were maintained in Dulbecco’s modified Eagle’s medium (10% fetal calf serum), and experiments conducted with passage 5 to 10 cells. Animal experimental procedures were in accordance with University of Tsukuba’s Regulations on Animal Experiments and Japanese Governmental Law No. 105.

Preparation of LDL

LDL was isolated from normal human blood by ultracentrifugation in the presence of EDTA as described previously.10 Oxidatively modified LDLs were formed by incubating LDL with 5 μmol/L CuSO₄ at 37°C, and the conjugated diene content determined by measuring absorbance at 234 nm. Lipid hydroperoxides in native (nLDL), moderately oxidized LDL (moxLDL), and highly oxidized LDL (oxLDL) were 40, 64, and 80 nmol/mg protein, respectively, and the relative electrophoretic mobilities of moxLDL and oxLDL (compared with native LDL) were 1.3 and 4.6, respectively.

Western Blot Analysis

Before electrophoresis, a marker dye and 2-mercaptoethanol were added to lysates, which were then fractionated by SDS-polyacrylamide gel electrophoresis and electrotransferred onto an Immobilon membrane (Millipore).20 Polyclonal rabbit antisera raised against purified rat HO-1, rat Prx I, and recombinant mouse A170 and Nrf2 were used. Nuclear pellets were prepared after cell lysis in 0.5% Nonidet P-40 containing buffer.26 Densitometric analysis was performed using NIH Image software.

Northern Blot Analysis

Total RNA was extracted from cells using RNeasy (QIAGEN), fractionated by electrophoresis and transferred to Zeta-Probe GT membranes (Bio-Rad). Membranes were probed with [³²P]-labeled cDNA prepared by Megaprime labeling (Amersham), using 18S rRNA cDNA as an internal control. As described previously,21 cDNA fragments for A170, HO-1, Prx I, and CD36 were prepared from Bluescript plasmid and used for hybridization.

Reverse Transcription–PCR Analysis

Oligonucleotide primers used for RT-PCR were as follows: 5'-TGTGCTAGACATTTGGGCAATACTG-3’ and 5’-CTTCTCTTCAAAGTAAAGCTGTTGGT-3’ for detecting mouse CD36 mRNA, 5’-AAAGATAGACACCATCACCC-3’ and 5’-GGCGACGTCAAAAAGTCTTCTCTC-3’ for mouse LOX-1 mRNA, 5’-TCTCTACCTCCTTGTTTG-3’ and 5’-GATGCGACTCATGGAATTCC-3’ for mouse macrophage scavenger receptor I (SR-A) mRNA, and 5’-TGAAAGGTCGAGTACCGATTGTGGT-3’ and 5’-CATGTGGGCCATGAGTCCACACAC-3’ for mouse GAPDH mRNA. Total RNAs isolated from macrophages treated with moxLDL or oxLDL for 24 hours were analyzed by RT-PCR using a QIAGEN OneStep RT-PCR kit. An aliquot of each RT-PCR mixture was electrophoresed on a 1.2% agarose gel and stained with Vistra Green (Amersham Pharmacia Biotech). The signal intensity of the RT-PCR products was determined using Fluoromager 595 (Amerham Pharmacia Biotech). The amounts of total RNA templates and cycle numbers for amplification were chosen in quantitative ranges determined by plotting signal intensities as functions of the template amounts and cycle numbers. Nucleotide sequences of the RT-PCR products were verified.

Oil Red O Staining

Mouse peritoneal macrophages were incubated for 24 hours at 37°C in 5% CO₂ in RPMI 1640 medium containing 10% FCS in the absence or presence of HNE (10 μmol/L) or moxLDL or oxLDL (200 μg protein mL⁻¹). Macrophages were washed twice, fixed in formalin, and stained with oil red O. The area of lipid-loaded macrophages was measured using a computerized MacSCOPE image analysis system (Mitani Corp).

Results

Oxidatively Modified LDL Activates Nrf2 in Peritoneal Macrophages

The effects of oxLDLs on nuclear accumulation of Nrf2 and stress gene expression in murine macrophages are shown in Figure 1. Cells were pretreated for 5 hours with nLDL, moxLDL, and oxLDL, and nuclear fractions were analyzed by immunostaining. Nuclear levels of Nrf2 were enhanced markedly after treatment with either moxLDL or oxLDL, whereas nLDL increased Nrf2 translocation marginally (Figure 1A, top). Increased Nrf2 levels induced by these agents were also detected in whole cell lysates (data not shown).

When effects of oxLDLs (5 hours) on A170, HO-1, and Prx I mRNA levels were determined in heterozygous mutant and Nrf2-deficient (homozygous mutant) macrophages, both moxLDL and oxLDL increased A170, HO-1, and Prx I.
mRNA levels (relative to 18S rRNA) in nrf2/H11001/H11002 cells, whereas nLDL had a negligible effect (Figure 1B). Basal expression of these transcripts was significantly lower in nrf2/H11002/H11002 compared nrf2/H11001/H11001 (or wild type, data not shown) macrophages, and oxLDLs caused only marginal increases in mRNA levels (Figure 1B). moxLDL only caused a concentration-dependent increase in A170, HO-1, and Prx I expression in nrf2/H11001/H11001 macrophages. In nrf2/H11002/H11002 cells, moxLDL only slightly enhanced A170 levels but did not increase expression of either HO-1 or Prx I (Figure 1C).

Effects of LDL Components on Induction of Stress Proteins

Because oxidized LDL contains lipid hydroperoxides, oxysterols, and aldehydes, we examined the effects of lyso-phosphatidylcholine (LPC), HPODE, 7-ketocholesterol, hexanal, malondialdehyde, 7β-hydroxycholesterol, and 4-hydroxy-2-nonenal (HNE). Treatment of macrophages for 8 hours with either LPC (50 μmol/L), 7-ketocholesterol (20 and 40 μmol/L), or hexanal (250 and 500 μmol/L) had no significant effect on A170, HO-1, and Prx I expression, whereas HPODE or malondialdehyde only slightly enhanced protein levels at 50 μmol/L (data not shown). 7β-Hydroxycholesterol increased A170, HO-1, and Prx I expression only at concentrations above 50 μmol/L (data not shown). We found that HNE was the most effective activator of Nrf2-mediated increases in stress protein mRNA and protein levels (Figures 1C and 1D). The estimated content of HNE in highly oxidized LDL is 114 nmol/mg LDL protein, corresponding to 17 μmol/L HNE when 150 μg protein/mL oxidized LDLs were added to culture media. These results suggest that HNE contained in oxidized LDL could be one of the activators of Nrf2.

4-Hydroxynonenal Activates Nrf2 and Stress Protein Expression in Peritoneal Macrophages

HNE increased nuclear translocation of Nrf2 after 1 to 5 hours (Figure 1A, bottom). HNE and diethylmaleate (DEM), a typical Nrf2-activating electrophilic agent, increased stress protein expression in nrf2/H11001/H11001 macrophages, with responses markedly attenuated in nrf2/H11002/H11002 cells (Figures 1C and 1D).
**Effects of Oxidatively Modified LDL and HNE in Murine Aortic Smooth Muscle Cells**

In contrast to our previous findings in macrophages, oxLDLs had a negligible effect on nuclear translocation and accumulation of Nrf2 in murine aortic smooth muscle cells (SMCs) (Figure 2A, top). In contrast, a rapid (1 to 3 hours), transient increase in nuclear Nrf2 levels was observed in SMCs treated with HNE (Figure 2A, bottom). When the effects of oxLDLs, HNE, and DEM on transcriptional activation of A170, HO-1, and Prx I were examined in nrf2−/− SMCs under standard culture conditions, relatively high basal mRNA levels were detected for Prx I and A170, whereas basal HO-1 mRNA levels were low (Figure 2B). Notably, nLDL, moxLDL, and oxLDL failed to increase mRNA (Figure 2B) or protein (data not shown) levels for A170, HO-1, and Prx I in nrf2−/− SMCs.

HNE and DEM significantly increased mRNA and protein levels of A170 and HO-1 in nrf2−/− SMCs, whereas Prx I expression was only slightly enhanced (Figures 2B and 2C). In nrf2−/− SMCs, neither HNE nor DEM affected A170, HO-1, and Prx I expression (Figure 2C). Thus, as in macrophages, increased stress protein expression in SMCs induced by HNE or DEM is largely dependent on activation of Nrf2, establishing a strong correlation between nuclear accumulation of Nrf2 and transcriptional activation of stress genes.

**Activation of CD36 Gene Expression by HNE and DEM in Murine Peritoneal Macrophages**

CD36 is the major scavenger receptor for the uptake of oxidatively modified LDL in macrophages. We previously isolated the mouse homologue of a CD36 cDNA clone and in the present study, Northern blot analysis showed that HNE and DEM increased CD36 mRNA levels in nrf2−/− but not nrf2−/− macrophages (Figure 3A, top). Immuno blot experiments established that HNE and DEM (8 hours) caused a 2.2-fold and 1.4-fold increase in CD36 protein levels in nrf2+/− macrophages, whereas upregulation of CD36 was minimal in nrf2−/− cells (Figure 3A, bottom). These findings suggest that increased CD36 expression in response to HNE and DEM is largely dependent on the activation of Nrf2.

Nrf2-Dependent Upregulation of CD36 Expression by Oxidatively Modified LDL

We could not detect upregulation of CD36 protein levels in macrophages treated with moxLDL or oxLDL for 8 hours (Figure 3A, bottom), and therefore examined concentration-dependent effects of moxLDL and oxLDL (50 to 200 μg protein mL−1) on CD36 expression at 8 and 24 hours in nrf2−/− macrophages. moxLDL and oxLDL dose-dependently increased CD36 levels only after 24 hours (Figure 3B). When effects of moxLDL and oxLDL (200 μg protein mL−1, 24 hours) were compared in nrf2−/− and nrf2−/− macrophages, induction of CD36 was significantly attenuated in Nrf2-deficient macrophages (Figure 3C). HNE markedly increased CD36 levels in nrf2−/− macrophages but was less effective in nrf2−/− macrophages, providing the first direct evidence that Nrf2 is important in the activation of CD36 gene expression in macrophages exposed to oxLDLs or HNE.

**PPAR-γ Activators Upregulate CD36 Expression in Nrf2-Deficient Macrophages**

PPAR-γ plays an important role in the induction of CD36 by oxidized LDLs. Under our experimental conditions, CD36 expression was upregulated in nrf2−/− macrophages by the PPAR-γ activators 15d-PGJ2 and rosiglitazone (Figure 3D), indicating that PPAR-γ activation of CD36 gene expression occurs via a signaling pathway distinct from Nrf2.

**Effects of Oxidatively Modified LDL on Expression of Other Scavenger Receptors**

We used quantitative RT-PCR to compare CD36, LOX-1, and SR-A mRNA levels in murine macrophages. In contrast to upregulation of CD36 in response to moxLDL and oxLDL (Figure 4A), mRNA levels for LOX-1 were downregulated whereas levels for SR-A remained unchanged (data not shown). Our findings are consistent with a previous report that increased uptake of oxLDL in macrophages is the result of increased expression of CD36 but not of the scavenger...
receptor SR-A type I or type II. 34 Oil red O staining of macrophages revealed that moxLDL significantly enhanced the accumulation of cholesterol in nrf2/H11001/H11001 cells, whereas the intensity of staining was much lower in Nrf2-deficient cells (see Figures 4B and 4C). HNE alone only slightly enhanced oil red O staining in both cell types. These results indicate that Nrf2-dependent upregulation of CD36 leads to an accumulation of cholesterol in macrophages treated with moderately oxidized LDL. However, experiments with CD36/H11002/H11002 peritoneal macrophages revealed that CD36 is not essential for activation of Nrf2 by oxLDL or HNE (data not shown).

Discussion

We report the first evidence that oxLDLs and HNE induce nuclear translocation of Nrf2 in murine peritoneal macrophages, resulting in an upregulation of the scavenger receptor CD36 and antioxidant stress proteins A170, HO-1, and Prx I. Our study establishes (1) Nrf2 as a novel signaling pathway involved in the regulation of CD36 gene expression in macrophages, (2) HNE as a potent activator of Nrf2 in both macrophages and SMCs, and (3) oxLDLs as effective activators of Nrf2 in macrophages but not in SMCs expressing negligible levels of CD36. In addition to identifying Nrf2 as a key transcription factor controlling antioxidant gene expression, our findings implicate Nrf2 as an important signaling pathway in atherosclerosis.

In macrophages, CD36 can be upregulated by oxLDL, 29,30 and previous studies have established an integral role for PPAR-γ in CD36 gene expression. 11,32 PPAR-γ modulates lipid homeostasis and antiinflammatory responses in macrophages 33 and is expressed at high levels in foam cells in atherosclerotic lesions. 11,35 PPAR-γ-deficient macrophages express low levels of both CD36 mRNA and protein, suggesting that PPAR-γ controls basal levels of CD36. 15 However, the latter study did not examine whether oxLDLs enhance CD36 expression in PPAR-γ-deficient macrophages. We have identified Nrf2 as a novel signaling pathway, distinct from PPAR-γ, that also upregulates CD36 expression in macrophages treated with oxLDLs. The following evidence supports this conclusion. First, HNE readily enters cells and is highly reactive with proteins and metabolized by enzymes such as aldo-keto-reductase. 28 Second, HNE does not activate PPAR-γ directly, 11,34 whereas rapid activation of Nrf2 by HNE leads to CD36 gene expression in macrophages (Figures 3A and 3C), implicating Nrf2 as an important transcription factor in the upregulation of CD36 in oxidative stress. Third, as PPAR-γ activators 15d-PGJ2 and rosiglitazone increased CD36 protein levels in both nrf2/H11001/H11001 and nrf2/H11002/H11002 macrophages treated for 8 or 24 hours in the absence (Ctr) or presence of 5 µmol/L 15d-prostaglandin J2 (dPGJ2) or 5 µmol/L rosiglitazone (Rosi).

Figure 3. Nrf2-dependent induction of CD36 expression in murine peritoneal macrophages. A, Top, CD36 mRNA levels in nrf2/H11001/H11001 and nrf2/H11002/H11002 macrophages treated for 5 hours with 20 µmol/L HNE or 100 µmol/L DEM. Bottom, Immunoblot comparing CD36 protein levels in nrf2/H11001/H11001 and nrf2/H11002/H11002 macrophages treated for 8 hours with 100 µg protein mL⁻¹ moxLDL, 20 µmol/L HNE, or 100 µmol/L DEM. B, Immunoblot showing CD36 expression in nrf2/H11001/H11001 macrophages treated either for 8 or 24 hours in the absence (Ctr) or presence of 50 to 200 µg protein mL⁻¹ moxLDL or oxLDL. C, Denatometric analysis of 24-hour treatment with moxLDL, oxLDL, or HNE (20 µmol/L) on CD36 expression in nrf2/H11001/H11001 and nrf2/H11002/H11002 macrophages. Values denote mean ± SEM of experiments in 3 to 5 different cell cultures. D, Effects of PPARγ activators on CD36 protein levels in nrf2/H11001/H11001 and nrf2/H11002/H11002 macrophages. Cells were treated for 24 hours in the absence (Ctr) or presence of 5 µmol/L 15d-prostaglandin J2 (dPGJ2) or 5 µmol/L rosiglitazone (Rosi).
HNE has been detected in rabbit and human atherosclerotic lesions, and Napoli et al. reported that a large percentage of all fetal atherogenic sites contained malondialdehyde-lysine and HNE-lysine epitopes.

Our findings in nrf2+/− macrophages indicate that moxLDL and oxLDL selectively upregulate CD36 mRNA expression (Figure 4A) but not the scavenger receptors LOX-1 or SR-A (data not shown). In contrast, in nrf2−/− macrophages oxLDLs and HNE fail to significantly increase CD36 levels (Figure 3C). We found multiple ARE-like sequences in the promoter region of murine CD36-encoding gene (GenBank accession No. AF434766) and further studies are required to identify the functional Nrf2-interacting ARE in this promotor region. A recent study indicates that AREs play a direct role in mediating the induction of glutathione synthesis by oxLDL, suggesting a role of Nrf2 in this response. The lack of induction of CD36 in nrf2−/− macrophages was associated with a reduced accumulation of cholesterol (Figures 4B and 4C), indicating that the Nrf2 signaling pathway plays a key role in mediating oxLDL uptake via CD36. Although there are limited reports that human cultured aortic SMCs express CD36, we could not detect CD36 expression in SMCs cultured from either nrf2+/+ and nrf2−/− mice (data not shown). These differences in CD36 expression may be important for comparisons of human and murine models of atherosclerosis.

Our study provides the first evidence that Nrf2, in addition to coordinating cellular defenses against electrophilic agents and reactive oxygen species, also plays an essential role in regulating CD36 expression. Figure 5 summarizes our experimental findings, and highlights that in murine macrophages oxLDLs and HNE lead to a rapid nuclear accumulation of Nrf2 and subsequent induction of CD36 and the stress proteins A170, HO-1, and Prx I. In murine SMCs, oxLDLs selectively upregulate CD36 mRNA expression and reactive oxygen species, also plays an essential role in mediating the induction of glutathione synthesis by oxLDL, suggesting a role of Nrf2 in this response. The lack of induction of CD36 in nrf2−/− macrophages was associated with a reduced accumulation of cholesterol (Figures 4B and 4C), indicating that the Nrf2 signaling pathway plays a key role in mediating oxLDL uptake via CD36. Although there are limited reports that human cultured aortic SMCs express CD36, we could not detect CD36 expression in SMCs cultured from either nrf2+/+ and nrf2−/− mice (data not shown). These differences in CD36 expression may be important for comparisons of human and murine models of atherosclerosis.

Future studies in vivo, using wild-type and Nrf2-deficient mice, should enable us to determine whether Nrf2 is expressed in atherosclerotic lesions, and whether this transcription factor modulates the progression of foam cell formation and atherosclerosis. Because Nrf2 has been implicated as a regulator of HO-1 expression and HO-1 mRNA and protein levels are increased in human atherosclerotic lesions, it seems likely that Nrf2 plays a role in atherogenesis. Identifying the intracellular signaling pathways modulating Nrf2 and PPAR-γ will provide important insights into their role in regulating vascular gene expression and may provide a basis for the design of therapeutic strategies to treat atherosclerosis.

and nrf2−/− macrophages (Figure 3D), this suggests that activation of PPAR-γ can occur in the absence of Nrf2.

HNE proved to be one of the most effective activators of Nrf2; however, we cannot exclude the possibility that other components of oxLDL may also have led to Nrf2 activation. Recent studies indicate that oxidized choline glycerophospholipid in oxLDL influences the binding of oxLDL to CD36. Earlier studies from this group provided convincing evidence that lipid accumulation and foam cell formation were significantly reduced in CD36−/− mice. Our preliminary studies with CD36−/− mice established that oxLDL and HNE induced activation of Nrf2 in murine macrophages is not dependent on CD36 (data not shown).

Kavanagh et al. reported that CD36 and PPAR-γ are differentially expressed in human monocytes in response to LDL oxidized to different degrees. Using oxLDL preparations similar to those in our study, this group established that moxLDL, but not oxLDL, enhanced DNA binding to PPAR-γ. Our experiments with nrf2−/− macrophages revealed that moxLDL still appears to increase, although not significantly, CD36 expression, whereas responses to oxLDL and HNE were abrogated (Figure 3C). We believe that HNE selectively activates Nrf2, resulting in a PPAR-γ-independent expression of CD36 in macrophages. In this context,
Figure 5. Nrf2-dependent activation of CD36 and antioxidant stress genes in murine peritoneal macrophages by oxidatively modified LDL (e.g., moxLDL or oxLDL) or HNE. Activation of the Nrf2 pathway in macrophages leads to an upregulation of CD36 expression and uptake of oxidatively modified LDL. HNE, one of the end products of lipid peroxidation, induces rapid activation (2 to 5 hours) of Nrf2 and enhanced expression of HO-1, Prx I, and A170. Induction of antioxidant stress genes via Nrf2 affords protection of cells against the toxicity of oxLDLs. In murine aortic smooth muscle cells, expressing low levels of oxLDL scavenger receptors, oxidatively modified LDLs cause a negligible activation of Nrf2, whereas HNE can rapidly activate antioxidant gene expression via Nrf2. Dotted lines denote intracellular signaling cascades leading to the activation of PPAR-γ and/or Nrf2.

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