We, and others, have shown that foam cell formation initiated by exposing macrophages to oxidized LDL (oxLDL) triggers the differential expression of a number of proteins. Specifically, our experiments have identified peroxiredoxin I (Prx I) as one of these upregulated proteins. The peroxiredoxins, a family of peroxidases initially described for their antioxidant capability, have generated recent interest for their potential to regulate signaling pathways. Those studies, however, have not examined peroxiredoxin for a potential dual-functionality as both cytoprotective antioxidant and signal-modulator in a single, oxidant-stressed system. In this report, we examine the upregulation of Prx I in macrophages in response to oxLDL-exposure, and its ability to function as both antioxidant enzyme and regulator of p38 MAPK activation. As an antioxidant, induction of Prx I expression led to improved cell survival following treatment with oxLDL or tert-butyl hydroperoxide. The improved survival coincided with a decrease in measurable reactive oxygen species (ROS), and both the increased survival and reduced ROS were reversed by Prx I siRNA transfection. Additionally, our data show that activation of p38 MAPK in oxLDL-treated macrophages was dependent on the upregulation of Prx I. Reduction of Prx I expression by siRNA transfection resulted in a significant decrease in p38 MAPK activation, while the upregulation of Prx I expression with either oxLDL or ethoxyquin led to increased p38 MAPK activation. These results are consistent with multiple roles for Prx I in macrophage-derived foam cells that include functionality as both an antioxidant and a regulator of oxidant-sensitive signal transduction.

A key event in the early stages of atherosclerosis is the internalization of oxidatively-modified low density lipoprotein (oxLDL) by intimal macrophages (1,2). This internalization generates lipid-laden macrophages, known as foam cells, that accumulate into fatty streaks and become potential sites for the continued development of advanced atherosclerosis (3,4). It is clear that these foam cells represent a unique macrophage phenotype that is distinctly proatherogenic. Relative to macrophages, foam cells have increased expression of various scavenger receptors that can enhance the lipid deposition process (5-7) and alter cholesterol trafficking by reducing reverse cholesterol transport (8,9). Further, foam cells upregulate cytokine production, which has been linked to both recruitment of additional monocyte/macrophages and smooth muscle cell proliferation (10-12). Overall, the net effect of these changes has all of the characteristics of a vicious cycle that increases the inflammatory response, ROS production, cell recruitment, and cell proliferation. These factors combine to drive progression of the lesion from the initial fatty streak toward the advanced atherosclerotic lesion.

We have recently examined changes in protein and mRNA expression that accompany foam cell formation and, among other effects, observed an upregulated antioxidant response (13). A unique component of our observations was the upregulation of members of the peroxiredoxin family, a set of ubiquitously-expressed peroxidases (13). The mammalian peroxiredoxin family consists of six proteins (Prx I-VI) expressed as unique gene products, with the capability to reduce hydrogen peroxide, lipid hydroperoxides and peroxynitrite (14-17). Prx I – IV are categorized as typical 2-Cys peroxiredoxins, distinct from the atypical 2-Cys (Prx...
V) and 1-Cys peroxiredoxin (Prx VI). In humans, Prx I – IV share >65% protein sequence homology, including the two conserved cysteines responsible for peroxide reduction. These 2-Cys peroxiredoxins function as homodimers, in which one conserved cysteine (Cys51 in Prx I) forms an intermolecular disulfide with the second conserved cysteine (Cys172 in Prx I) upon reduction of a substrate. The disulfide is reduced by the electron donor thioredoxin, re-activating peroxiredoxin antioxidant capability. At elevated oxidant levels, Prx I - IV can be inactivated through the over-oxidation of the active site cysteine to sulfinic (Cys-SO2) or sulfonic acid (Cys-SO3) (18,19). The Cys-SO2 modification is reversed by sulfiredoxin in an ATP-dependent reductive mechanism (20,21). The significance of this reversible inactivation as a means to regulate intracellular peroxide levels has generated interest in peroxiredoxin as a mediator of H2O2-regulated signaling pathways (22-24).

The findings presented in this report focus on Prx I, a basic (pI 8.3) 22 kDa protein localized to the cytosol. Prx I is upregulated in a variety of cell types following exposure to oxidative stress. Upregulation of Prx I in response to H2O2 was first demonstrated in mouse peritoneal macrophages (25), and in cultured vascular smooth muscle cells following exposure to oxLDL (26). Further studies characterized the upregulation of Prx I resulting from exposure to ionizing radiation (27,28), hyperoxia (29), and 4-hydroxy-2-nonenal (30). Additionally, elevated levels of Prx I have been discovered in several types of cancer, including lung (31,32), breast (33) and pancreatic cancer tissues (34).

The induction of the peroxiredoxins is generally related to a cellular antioxidant response. However, recent findings suggest alternative functionality for the peroxiredoxin family, including the ability to modulate various signaling pathways. Prx II, for example, is believed to regulate peroxide levels that would otherwise inhibit phosphatases such as PTEN (24). PTEN converts the signaling molecule PIP3 to PIP2, inhibiting the downstream activation of Akt kinase. Thus, increased Prx II activity is able to protect and support increased PTEN activity, and prevent the downstream phosphorylation of Akt targets. Additionally, Prx II can regulate H2O2 levels produced in response to TNF-α, and thus limit the activation of the JNK and p38 MAPKs in response to increased peroxide levels (35). One report invoking a similar role for Prx I in intracellular signaling has also been made. In those experiments, the Schizosaccharomyces pombe 2-Cys peroxiredoxin Tpx1, the yeast homolog for mammalian Prx I, was shown to regulate the peroxide-induced activation of Sty1, the yeast homolog for mammalian p38 (36). The regulation occurs through a direct association of Tpx1 with Sty1, forming an intermolecular disulfide that protects Sty1 from oxidant-induced inactivation (36). A parallel role for mammalian Prx I in p38 regulation in macrophages would be significant, considering the downstream effects of p38 activation (37,38).

This report characterizes the functional significance of Prx I and its induction in macrophage foam cells. The oxLDL-induced formation of foam cells from macrophages is shown to result in the upregulation of active, rather than oxidatively-inactive, Prx I. By applying methods to either induce or inhibit Prx I protein expression, our data show that Prx I upregulation prior to foam cell formation promotes cell survival, and that the protective effect of Prx I corresponds directly to its ability to reduce reactive oxygen species. Additionally, the role of Prx I as a modulator of p38 activity is established by demonstrating the dependence of p38 phosphorylation on Prx I expression. Our results are consistent with a dual role for Prx I, in which 1) induced expression can provide antioxidant functionality by reducing cytotoxic levels of ROS, and 2) the extent of Prx I expression can govern the level of p38 MAPK activation.

**EXPERIMENTAL PROCEDURES**

**Materials** – siRNA and siPORT amine transfection reagent were obtained from Ambion Inc. (Austin, TX). Rabbit polyclonal antibody for Prx I was obtained from BioMol Int. (Plymouth Meeting, PA). Rabbit polyclonal antibody for Prx I-SO3 and mouse monoclonal antibody for GAPDH were obtained from Abcam (Cambridge, MA). Rabbit polyclonal antibodies for p38,
phosphorylated p38 (Thr180/Tyr182), Akt and phosphorylated Akt (Ser473) were obtained from Cell Signaling (Danvers, MA). For detection of ROS, 5-(and-6)-chloromethyl-2′7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA), was obtained from Molecular Probes (Eugene, OR). Ethoxyquin, hydrogen peroxide and tert-butyl hydroperoxide were purchased from Sigma-Aldrich (St. Louis, MO). The p38 MAPK inhibitor SB 203580 was obtained in a 1 mg/mL DMSO solution from EMD Biosciences (San Diego, CA).

Lipoprotein Preparation - Fresh human plasma was obtained from the Cleveland Clinic Blood Bank and the LDL isolated by differential ultracentrifugation (1.019 < d < 1.063 g/ml) (39). This LDL preparation, in NaBr solution containing 0.02% EDTA, was dialyzed against 0.9% NaCl, 0.02% NaN3, 0.02% EDTA (pH 7.4) and stored in the dark at 4°C (40). Oxidatively-modified LDL was prepared as previously described (13). Oxidative modification was assayed with the TNBS assay of free amines, which typically resulted in 40% modified amines compared to 3% modified amines when LDL was dialyzed in TBS only.

Cell Culture Conditions - J774A.1 murine macrophages were obtained from American Type Culture Collection (ATCC TIB 67) and maintained in culture media (DMEM containing 10% fetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin) at 37°C in 5% CO2. Cells were split every 2 – 3 days when ~80% confluence was reached, and discarded after 20 passages.

siRNA Inhibition of Prx I - Approximately 5x10^5 cells were plated in 60mm tissue-culture treated plates and left to adhere overnight at 37°C in 5% CO2. For each 60mm plate, 13 μL of siPORT Amine Transfection Reagent (Ambion Inc., Austin, TX) was incubated with 200 μL Opti-Mem reduced-serum medium for 10 minutes. Annealed double-strand siRNA (20 μM stock) was added to a separate aliquot of 200 μL Opti-Mem to give a final siRNA concentration of 30 nM. The transfection agent and the siRNA mixtures were combined and incubated for 10 minutes at room temperature. Media in the 60 mm culture plates was changed to 4 mL DMEM + 10% FCS only (no antibiotics), and the siRNA-transfection agent complexes were added drop-wise. Transfection conditions were maintained for 16 – 24 h. The Prx1 siRNA sequence, obtained from the online Ambion siRNA library (ID #68674), targeted exon 4 and was as follows: sense - GGAUUAUGGAGUCUUAAAGtt, antisense - CUUUAAGACUCCAUAAUCCtg. Scrambled and reverse siRNA sequences were designed and used as negative controls. All siRNA were obtained in lyophilized, annealed form, resuspended in DEPC ddH2O to a stock concentration of 20 μM and stored at -30°C in 100 μL aliquots.

Cell Toxicity Assay - Approximately 2x10^4 cells per well were seeded into 24-well tissue culture treated plates and incubated overnight at 37°C in 5% CO2. For oxLDL-induced toxicity, cells were treated for up to 48 hours with 10 to 100 μg/mL oxLDL. For tert-butyl hydroperoxide-induced toxicity, cells were treated with 10 to 200 μM tert-butyl hydroperoxide in DMEM for 2 hours, followed by a 48 hour incubation in normal culture media. Cell survival was assayed with the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison WI) as previously described (13). Results were calculated from an average of three independent treatments, and experimental error is expressed as ± 1 standard deviation.

Flow Cytometric Assay of Reactive Oxygen Species - Reactive oxygen species (ROS) were assayed using a Guava EasyCyte Flow Cytometer and the ROS-detecting agent CM-H2DCFDA. Lyophilized CM-H2DCFDA (50 μg) was suspended in DMSO to a stock concentration of 1 mM and added to pre-warmed Hank’s Balanced Salt Solution without phenol red (HBSS) to a working concentration of 10 μM. Cells were washed with HBSS, covered with the CM-H2DCFDA/HBSS solution (typically 1.5mL per well in a 6-well plate), and incubated at 37°C/5% CO2 for 10 minutes. Cells not exposed to CM-H2DCFDA were included to assay the autofluorescent background initiated by experimental conditions. Following the CM-H2DCFDA incubation, the media was removed and cells were washed with HBSS, then harvested by scraping in 1 mL HBSS, centrifuged at 1000 rpm in an Eppendorf Microfuge, and washed once with PBS. Cells were then fixed in 3.7% formalin for 15
minutes at room temperature. The cells were pelleted by centrifugation, washed once with PBS, and resuspended in 500 μL PBS for further analysis. For flow cytometric analysis, 5000 events were counted within the gate parameters for each sample. Acquisition parameters were as follows: forward gain = 2x, side scatter = 330 V, forward scatter threshold = 215, green channel = 552 V, red channel = 524 V, yellow channel = 533 V. Gate parameters were set to X1 = 251, X2 = 2154, Y1 = 3857, Y2 = 574. A 5000-count acquisition required approximately 20 μL sample and 30 seconds at a flow rate of 0.59 μL/sec. Background fluorescence measured in cells without CM-H2DCFDA was subtracted from values obtained for the respective CM-H2DCFDA-treated samples. This background was most significant when assaying oxLDL-treated macrophages, and ranged from 10 – 30% of the total measured fluorescence. The background for all other treatments was less than 10%. Results are normalized with respect to ROS measured in untreated control cells, and expressed as relative n-fold change in ROS. Results were calculated from an average of three independent treatments, and experimental error is expressed as ± 1 standard deviation.

2D Western Blot Analysis of Oxidized Prx1 - Approximately 10^6 cells were seeded onto 100mm tissue-culture treated plates and incubated overnight. Cells were treated with oxLDL, tert-butyl hydroperoxide or an equivalent volume of buffer (20 mM Tris-buffered saline, pH 7.8), incubated for a given time period, harvested by scraping in cold DMEM, and washed once in sterile PBS. Cell pellets were boiled in SDS-lysis buffer (25 mM Tris, pH 7.5; 2.5 mM MgCl2; 0.5% SDS) for 5 minutes, cooled to room temperature, and treated with 50 μg/mL DNase I and RNase A for 15 minutes. The protein concentration was determined using a SDS-compatible method (Bio-Rad DC Protein Assay Kit), and 250 μg total protein was precipitated in acetone (80% v/v) overnight. The dried protein precipitate was prepared and analyzed by 2D SDS-PAGE as previously described (13). The 2D gel was immediately incubated in Novex Tris-Glycine Transfer Buffer + 10% methanol for 10 minutes. The gel was transferred to PVDF in a Bio-Rad Criterion Gel Transfer Chamber for 1 hour at 100V, blocked in 2% non-fat dry milk and incubated overnight at 5°C in Prx I rabbit polyclonal antibody (1:2000). Western blots were completed with a 1 hour incubation in HRP-conjugated donkey anti-rabbit secondary antibody, followed by enhanced ECL chemiluminescent detection. The membranes were stripped and re-probed with Prx I-SO2/-SO3 rabbit polyclonal antibody (1:1000). All samples were analyzed in parallel by 1D Western blot for GAPDH to confirm equal loading.

p38 MAPK inhibition – SB 203580 was used to inhibit p38 activity. SB 203580 (10 μM) was added to cell culture for 30 minutes prior to experiment. An equivalent volume of anhydrous DMSO was added to control cultures without SB-203580. The inhibitor was left in the culture for the duration of the experiment.

RESULTS

OxLDL-treatment Increases Prx I Expression - Previously, our group used a proteomic approach to study changes in protein expression that occur as macrophages become loaded with oxidized LDL to become foam cells (13). The 2D SDS-PAGE gel shown in Figure 1A represents a typical set of protein bands observed from the J774 murine macrophages used in these experiments. The gel covers the pI range of 3 to 10 and was obtained from a whole cell homogenate. More detailed views of two such 2D gels show a band with a significant increase in expression in the macrophages treated with 50 μg/mL oxLDL for 24 hours (Fig. 1C) compared to untreated macrophages at the same time-point (Fig. 1B). This band was cut from the gel, digested with trypsin, and identified by tandem mass spectrometry as Prx I (NCBI nr accession number 547923). The oxLDL-induced upregulation of Prx I seen in the 2D electrophoresis experiments was examined more closely by Western blot analysis. Figure 2A shows that Prx I expression is not changed in macrophages left untreated or treated with native LDL (50 μg/mL) for 24 or 48 hours. However, exposure of the macrophages to 50 μg/mL oxLDL results in a significant upregulation of Prx I protein, with continued increases from 24 to 48 hours. The oxLDL-induced upregulation was also dose-dependent, increasing with oxLDL...
concentration over a range from 10 to 50 μg/mL oxLDL (Fig. 2B). Concentrations greater than 50 μg/mL could not be tested due to increasing toxicity of the treatment.

Additional experiments were carried out to verify that the increase in Prx I was in a native (non-oxidized) form. Under certain oxidant-treatment conditions, the active site cysteine of Prx I can be over-oxidized to a sulfenic (SO₂) or sulfonic (SO₃) acid, inactivating the reductase function of the enzyme (18). The oxidatively-inactivated forms of Prx I can be detected on a 2D SDS-PAGE gel by an acidic band shift associated with sulfenic or sulfonic acid formation at the affected cysteine residues, or by an antibody specific to oxidized peroxiredoxin (Prx I-IV). Figure 3 shows a combination of 2DE and Western blot analysis used to verify that the increased expression of Prx I results in the functionally-active, non-oxidized form of the protein. For this experiment, the J774 macrophages were exposed to tert-Butyl hydroperoxide (t-BOOH) (100 μM) or oxLDL (50 μg/mL) for 24 hours. t-BOOH was chosen as a positive control based on its known ability to oxidize Prx I (41).

The antibody used to detect oxidized, inactive Prx I is specific to both the sulfonic (Cys-SO₃) and sulfenic (Cys-SO₂) modifications of Prx I. These forms are detected as separate bands that are differentiated by the acidity of these modifications. Accordingly, the left-most band in each panel of Figure 3 represents the Cys-SO₃ modification and the center-band represents the Cys-SO₂ modification of Prx I. Both of the oxidized Prx I forms were detected by the oxPrx I antibody (Figs. 3D, E, F), further supporting these identifications. The active, unmodified Prx I has a calculated pI of 8.3, which corresponds to the right-most band position.

After 24 hours of oxLDL-treatment, the active form of Prx I is upregulated by oxLDL-treatment (Fig. 3C) with respect to the untreated Ctl macrophages (Fig. 3A). In both the t-BOOH and oxLDL treatments, the center bands representing inactive, SO₂-modified Prx I were increased, consistent with the ability of both treatments to modify Prx I. These 2D data, combined with the data shown in Figure 2, clearly demonstrate that the oxLDL-induced upregulation of Prx I produces a significant increase in the active, unmodified form of the protein.

**Induction and Inhibition of Prx I Protein Expression** - In order to characterize the role of Prx I in foam cell survival, methods were developed to induce and inhibit Prx I protein expression. Prx I-induction was achieved by treatment with ethoxyquin, a small molecule capable of inducing the expression of phase II detoxifying proteins (42). Figure 4 shows a series of Western blots used to determine changes in Prx I expression that occur with different combinations of ethoxyquin and oxLDL treatment. Ethoxyquin induces Prx I protein expression in a dose-dependent manner (Fig. 4A). Figure 4B demonstrates the combined effect of ethoxyquin pre-treatment for 24 hours, followed by oxLDL treatment for 24 hours. In macrophages treated with 25 μg/mL oxLDL, ethoxyquin pre-treatment gave an increase in Prx I expression compared to oxLDL-treatment alone. When treated with 50 μg/mL oxLDL, however, the extent of Prx I upregulation is similar in macrophages with and without ethoxyquin pre-treatment. Thus, ethoxyquin may enhance oxLDL-induced expression of Prx I under certain conditions, but this increase can be saturated at higher concentrations of oxLDL.

Conversely, Prx I expression was knocked-down by transfection with a Prx I-specific siRNA (Prx I-KD macrophages). This siRNA treatment gave an effective reduction in Prx I expression at concentrations greater than 30 nM and did not affect the other major cytosolic peroxiredoxin, Prx II (Fig. 5B). Negative controls, including transfection reagent-only, scrambled and reverse sequence siRNA did not affect the expression of Prx I or Prx II (Fig. 5B). Importantly, the siRNA treatment also effectively inhibited the oxLDL-induced increase in Prx I expression (Fig. 5C).

**Effect of Prx I Induction and Inhibition on OxLDL-Induced Cytotoxicity** - Prx I is classified as an antioxidant enzyme based on its ability to catalyze the reduction of hydrogen peroxide and lipid hydroperoxides. Therefore, a likely functional effect of Prx I induction would be the protection of macrophages from the cytotoxicity of oxLDL. This cytoprotective potential was characterized with the induction and inhibition of Prx I expression prior to oxidant exposure, using both oxLDL and t-BOOH as
probes for the protective effect. The t-BOOH treatment was added as a model of lipid hydroperoxides, which are a component of oxLDL (43,44) and substrates for Prx I (45).

For these cytotoxicity experiments, four groups of macrophages were tested, including a control group and three groups with altered Prx I expression; the results are shown in Figure 6. The control macrophages (Ctl) were transfected with a reverse sequence siRNA that had no effect on Prx I expression. Prx I expression was knocked down with a 24 hour transfection of Prx I siRNA (Prx I-KD) or increased with a 24 hour treatment with ethoxyquin (+Ethx). Finally, the Prx I component of the ethoxyquin effect was tested with a combination of ethoxyquin treatment and Prx I siRNA transfection (Prx I-KD, +Ethx). The four groups of cells were then exposed to increasing concentrations of t-BOOH and cell survival was determined (Fig. 6A). Knock down of Prx I expression in the Prx I-KD macrophages resulted in a significant decrease in cell survival following t-BOOH treatment compared to control macrophages, consistent with a sensitizing effect in the Prx I knockdown. In contrast, Prx I induction by the ethoxyquin pre-treatment was protective and increased cell survival following t-BOOH exposure. Finally, the protective effect produced by the ethoxyquin treatment was partially reduced when combined with Prx I expression knockdown (the Prx I-KD, +Ethx treatment). These data demonstrate that Prx I contributes significantly to the ethoxyquin-induced cytoprotective response.

The pattern of cytotoxicity observed with oxLDL treatments showed an intriguing difference (Fig. 6B). The protective effect of the ethoxyquin treatment (+Ethx vs. Ctl) was still observed with oxLDL-induced toxicity and a component of this protection was reversed by the Prx I knockdown (Prx I-KD, +Ethx vs. Ctl); consistent with the effects seen in the t-BOOH exposure. Thus, the ethoxyquin-induced upregulation of Prx I prior to the treatment with oxLDL or the oxLDL-component-model t-BOOH decreases sensitivity to oxidant-induced cytotoxicity. In contrast to t-BOOH-exposure, however, no difference was seen in cell survival for the Prx I knockdown relative to the control macrophages (Prx I-KD vs. Ctl). Therefore, these data show that while the upregulation of Prx I that occurs during macrophage foam cell formation can protect against selected oxidants, the non-stimulated level of Prx I in macrophages do not have measurable effects on oxLDL-induced toxicity.

As an antioxidant, the cytoprotective effect of Prx I could be associated with its ability to reduce reactive oxygen species (ROS). To test this function, t-BOOH and oxLDL-induced ROS levels were assayed using the fluorescent ROS indicator CM-H2DCFDA to determine if changes in intracellular ROS correspond to the changes in oxidant-induced toxicity seen with Prx I induction and inhibition. These data are shown in Figure 7. The control macrophages were transfected with reverse sequence siRNA (Ctl). Treatment with either t-BOOH or oxLDL gave corresponding dose-dependent increases in the relative ROS measurements. In Prx I-KD macrophages, a significant increase in ROS production was observed with both oxidants. Conversely, Prx I induction with 50 μM ethoxyquin (+Ethx) gave a significant decrease in ROS production and a component of the reduction was reversed when combined with Prx I knockdown (Prx I-KD, +Ethx). As a group, these data show that changes in ROS detection correspond directly to Prx I knockdown or induction, including the demonstration that Prx I contributes to the reduction in ROS observed with ethoxyquin treatment.

Prx I regulation of p38 MAPK Activation - Prx I was tested for its ability to regulate the activation of p38 MAPK stimulated by various methods. Control and Prx I-KD macrophages were exposed to 100 μM H2O2 for up to 2 hours and analyzed by Western blot to determine expression of Prx I, phosphorylated and total p38 MAPK (Fig. 8A). In control macrophages, phosphorylation of p38 MAPK increased over time as total Prx I and p38 MAPK remained constant. In Prx I-KD macrophages, phosphorylation of p38 MAPK was negligible with respect to control macrophages until 2 hours after H2O2 exposure. At 2 hours, phosphorylation of p38 MAPK was still significantly decreased in the Prx I-KD macrophages compared to controls. These data are the first confirmation that the mammalian Prx I expression directly regulates p38 MAPK activation as seen with the yeast homologs Tpx1 and Sty1 (36).
The next experiment sought to determine if the differential regulation of Prx I would directly regulate the extent of p38 MAPK activation. Control and Prx I-KD macrophages were treated with 50 µM ethoxyquin to induce Prx I expression (Fig. 8B). Phosphorylation of p38 MAPK increased with ethoxyquin-induced Prx I upregulation. In Prx I-KD macrophages, expression of Prx I was inhibited in both the 0 and 50 µM ethoxyquin-treated macrophages, and the corresponding phosphorylation of p38 MAPK was negligible in each. Akt activation was also assayed due to the pro-survival nature of the Akt pathway. Ethoxyquin-treatment did not result in an increase in activated Akt, or in a change in Akt expression. Thus, the induction of Prx I by ethoxyquin creates conditions under which p38 MAPK activation is increased, while Akt activation remains constant.

Finally, the Prx I-regulated activation of p38 MAPK was tested under conditions of macrophage foam cell formation. Control and Prx I-KD macrophages were exposed to 10, 25 or 50 µg/mL oxLDL for 24 hours. Prx I was upregulated in a dose-dependent manner, while total p38 MAPK expression remained constant. In control and Prx I-KD macrophages, phosphorylation of p38 MAPK increased with oxLDL concentration. However, in Prx I-KD macrophages, phosphorylation of p38 MAPK was significantly reduced in parallel with the near-complete inhibition of Prx I. Akt activation remained unchanged with oxLDL-treatment. At this time, it is not clear if this modest amount of p38 MAPK activation is due to the small amount of residual Prx I expression or the presence of other, non-Prx I-dependent p38 MAPK activation pathways. Nonetheless, these data present a clear role for Prx I as a necessary component for maximal p38 MAPK activation.

The observed increase in p38 MAPK activation with Prx I induction raises the possibility that this activation is responsible for the increase in antioxidant activity, rather than the Prx I induction, either directly or by downstream effects. To test this possibility, the ability of Prx I to decrease ROS levels was examined under conditions where Prx I was induced but p38 MAPK activity was inhibited (Fig. 9). Macrophages were treated with 50 µM ethoxyquin to induce Prx I or left untreated for 24 hours. The compound SB-203580 was used to inhibit p38 MAPK, and the samples exposed to 200 µM t-BOOH for 2 hours and assayed for ROS levels. Ethoxyquin-exposed macrophages, which express increased levels of Prx I, showed the expected decrease in ROS compared the untreated controls, consistent with the antioxidant activity of the induced Prx I, but the p38 MAPK inhibition did alter this effect. Therefore, the decrease in ROS levels that results from Prx I induction (Figs. 7 and 9) is not dependent on p38 MAPK activity.

**DISCUSSION**

The oxLDL-induced transition from macrophage to foam cell is accompanied by the differential expression of a significant set of proteins, including inflammatory mediators (46-48), antioxidant proteins (30,49), and scavenger receptors (50,51). Previous research conducted by our laboratory characterized differential protein and mRNA expression that occurs during macrophage foam cell formation (13). Our data identified members of the peroxiredoxin family, a class of peroxidases that are upregulated under various conditions of oxidative stress, including hyperoxia (29), ischemia/reperfusion (52), radiation (28), and exposure to oxidant-generating reagents (26). A comparison of 2D SDS-PAGE gels showed that Prx I is upregulated in J774 murine macrophages following treatment with oxLDL under conditions that generate lipid-loaded macrophage foam cells in vitro (13). Despite work by other laboratories showing the upregulation of peroxiredoxins under various treatment conditions, studies of the their oxLDL-induced upregulation is limited (26,30). The data presented here fully demonstrate the upregulation of Prx I with oxLDL treatment. Through Western blot analysis, upregulation of Prx I was induced by oxLDL, but not native LDL, and both the dose-dependent and time-dependent characteristics were determined.

While the oxLDL-induced increase in Prx I expression is quantitatively significant, the data does not indicate whether Prx I is in its active form. Therefore, an additional step in the evaluation of...
expression was required to demonstrate that the redox state of Prx I was consistent with the fully functional form. The reducing function of the 2-Cys peroxiredoxins, including Prx I, is a two-step process that includes a sulfenic acid intermediate state (Cys-SOH) en route to an intermolecular disulfide across its homodimer structure. The disulfide is reduced by thioredoxin, returning functionality to peroxiredoxin. Recent attention has focused on the reversible inactivation of Prx I – IV caused by over-oxidation of the active site cysteine. In a mechanism proposed by Rhee and co-workers, the sulfenic acid intermediate can undergo further oxidation to a sulfinyl (Cys-SO₂H) or sulfonic acid (Cys-SO₃H) that can not be reduced by thioredoxin (18,19). This over-oxidation step is a particular concern for the oxidant-induced upregulation of peroxiredoxin, because the conditions that lead to increased peroxiredoxin expression can also oxidatively-inactivate newly expressed peroxiredoxin. The higher oxidation state is not detectable in 1D SDS-PAGE analyzed by Western blot, because both the active and inactive forms of peroxiredoxin run at the same position in the gel. The modification is, however, readily apparent by the acidic shift on a 2D Western blot of Prx I, and confirmed when the Western is re-probed using an antibody specific to the Cys-SO₂ and Cys-SO₃ species (oxPrx I antibody). It should be noted that the oxPrx I antibody identifies the oxidized species of all typical 2-Cys peroxiredoxins (Prx I – IV). Because Prx I and Prx II are comparable in molecular weight, the use of this antibody on a 1D gel is not sufficient to specifically identify inactive Prx I. However, due to a significant difference in isoelectric point, Prx I and Prx II run at distinct locations on a 2D SDS-PAGE gel. Therefore, the conditions of this experiment are uniquely suited for identifying active vs. oxidatively-inactive Prx I.

While past studies have applied t-BOOH (53) or H₂O₂ (18) as an oxidative stress, there is currently no evidence for the oxidative inactivation of Prx I in macrophage foam cells due to an oxLDL treatment. The presence of active, upregulated Prx I was detected by the combination of 2D SDS-PAGE with Western blot analysis described above. Both untreated and oxidant-treated macrophages contain detectable amounts of modified Prx I, implying that Prx I may be modified by endogenous oxidants under normal culture conditions. The increased abundance of Cys-SO₂ bands detected with both the oxLDL and t-BOOH- treatments shows that oxLDL is able to oxidized Prx I in a manner that is comparable to t-BOOH (54). More importantly, however, these 2D experiments also show that the increase in this modification is small and confirm that the oxLDL-induced upregulation of active Prx I produces a substantial increase in the active form.

The next stage of this investigation sought to determine the functional significance of Prx I upregulation by investigating the effects of Prx I induction on foam cell survival under conditions of t-BOOH- and oxLDL-induced cytotoxicity. These experiments were based on the protective effects of the peroxiredoxins, including Prx I, described in other cells treated with other types of cytotoxins, including oxidants (27,55-57). Our data show that inducing the expression of Prx I with ethoxyquin increased cell survival for both t-BOOH- and oxLDL-induced toxicity. In each case, the increased survival was attenuated by the Prx I siRNA transfection, thus confirming a significant role of Prx I induction in the enhanced survival.

It was notable, however, that the direct knockdown of Prx I sensitized the cells to t-BOOH-induced toxicity but had no effect on the oxLDL-induced toxicity. There are at least two possible explanations for this difference. First, the sensitization to t-BOOH-induced toxicity by Prx I knockdown may be related to the relatively uniform nature of this oxidative stress. Lipid peroxidation products, modeled by t-BOOH, are prototypical substrates for peroxiredoxin. Therefore, the decrease in Prx I activity would directly diminish the metabolism of the t-BOOH and give a corresponding reduction in cell survival. Oxidatively-modified LDL, however, is a heterogeneous mixture of oxidants and oxidation by-products, including lipid hydroperoxides, aldehydes, and oxysterols (58-60). Prx I may aid in the reduction of lipid peroxidation products generated by or contained in oxLDL, but other toxic components of oxLDL would remain unaffected. Second, a number of other effective antioxidants and antioxidant enzymes are present in the untreated cells, giving an element of redundancy to the antioxidant system. In this case, the decreased
antioxidant activity caused by Prx I knock-down would not be a significant loss to the overall antioxidant capability of the cell and would not produce a measurable change in the cytotoxicity of oxLDL. However, the increase in Prx I expression induced prior to the oxidant exposure appears to give a significant increase to the total antioxidant capacity, which translates to an increase in cell survival. This positive effect of pre-induced factors is a common theme in cytoprotection, i.e. the induction of cellular stress response results in increased protection during a subsequent cellular stress (13,61-63).

Parallel experiments examined the ability of the differentially-expressed Prx I to affect intracellular ROS. Inhibiting Prx I expression resulted in increased ROS in both t-BOOH and oxLDL-treated macrophages compared to controls. Conversely, increasing Prx I expression with ethoxyquin prior to oxidant exposure decreased the ROS levels relative to control macrophages that were not pre-treated with ethoxyquin. When the cells were treated Prx I siRNA prior to ethoxyquin pre-treatment, ROS levels were increased to the levels seen in the untreated controls.

A few details of the ROS detection, which influence the interpretation of these results, deserve attention. First, not all oxidants may target CM-H₂DCFDA for oxidation with equal affinity. While the oxidation of dichlorofluorescin (DCFH) analogs by H₂O₂ is well characterized (64-66), some data suggests that singlet oxygen does not oxidize DCFH to the fluorescent moiety, dichlorofluorescein (DCF) (67). However, while some oxidants may not target DCFH analogs for oxidation, they can still produce downstream oxidants for which DCFH is a substrate. A second important consideration involves the presence of endogenously-produced ROS in addition to the exogenous oxidants introduced by t-BOOH and oxLDL (68-70). A sub-lethal oxLDL exposure induces NADPH-oxidase to produce endogenous H₂O₂ (71), which has gained attention for its role in regulating signal transduction. As a result, the ROS assayed in these experiments include those introduced exogenously by t-BOOH and oxLDL, and those induced endogenously as a response to cellular stress. With these considerations in mind, there remains a direct correlation between the induction of Prx I, the generation of ROS and the resulting cytotoxicity. These data show that the induction of Prx I prior to foam cell formation provides a protective role through its ability to reduce ROS levels generated during oxLDL-uptake. These results provide a mechanistic link between the cytoprotective effects of Prx I induction and the enzymatic activity of Prx I to metabolize, and thereby decrease, the amounts of intracellular ROS. In both cases, pre-treating the cells with ethoxyquin results in decreased oxidant-induced cytotoxicity and a corresponding decrease in oxidant-induced ROS. When the induction of Prx I is eliminated from the effect of ethoxyquin treatment, both toxicity and ROS levels increase.

Overall, these data describe an oxidant-protective role for Prx I in macrophage-derived foam cells when the induction of Prx I occurs prior to oxLDL exposure. Such a situation may occur if macrophages endure chronic exposure to sub-lethal levels of oxLDL prior to foam cell formation, thus leading to enhanced foam cell survival (69,70). Although several roles have been attributed to Prx I, this antioxidant functionality was the key to its discovery and remains the most commonly associated role (72,73). At the same time, however, the lack of a direct effect on oxLDL-induced toxicity in the Prx I-KD macrophages is also consistent with other activities for Prx I, beyond the toxicity-protective antioxidant effects.

Therefore, a subsequent series of experiments were carried out to look at the role of Prx I in oxidant-dependent signaling systems. Since the initial report describing the ability of Prx I to associate with and inhibit c-Abl kinase activity (74), the interest in the peroxiredoxins as signal regulators has steadily increased. Prx I has been described as a tumor suppressor due to its ability to associate with a region of the c-Myc regulatory domain and inhibit c-Myc-mediated transformation (75). However, most of the data describing signal regulation by peroxiredoxin has focused on a different cytosolic peroxiredoxin, Prx II. A role for Prx II as an endogenous ROS scavenger that prevents the oxidant-induced deactivation of PTEN phosphatase has been reported (24). By positively regulating PTEN function, Prx II prevents the downstream activation of pro-survival Akt kinase targets. Both
PTEN, and Prx II by association, are described as tumor suppressors for this regulatory role. Additionally, Prx II has been shown to reduce endogenous H$_2$O$_2$ produced in response to TNF-alpha signaling, and thus limit the activation of the JNK and p38 pathways while stimulating ERK activation (35). Regulation of the p38 MAPK pathway is directly linked to pro-atherogenic factors. Exposing cells to oxLDL has been shown to activate the p38 MAPK pathway and lead to oxLDL-induced toxicity (37). Additionally, oxLDL-induced p38 MAPK activation has been linked to the downstream release of the pro-inflammatory cytokines TNF-$\alpha$ and IL-1$\beta$ (76).

Recently, it was shown that Tpx1, the yeast homolog of Prx I, protects Sty1, the yeast homolog of p38 MAPK, from oxidative inactivation (36), but a similar role for Prx I has not been presented in mammalian cells. Further, oxidative conditions that upregulate Prx I have not been studied to determine the effect of differential Prx I expression on p38 MAPK activation. Therefore, p38 MAPK activation was assayed to determine if the upregulation of Prx I during macrophage foam cell formation could regulate p38 MAPK activation.

The initial use of H$_2$O$_2$ to induce activation of p38 MAPK was based on the previous results from yeast (36). In our experiments, the reduced expression of Prx I by siRNA knock-down coincided with a decrease in H$_2$O$_2$-induced p38 MAPK phosphorylation. Conversely, ethoxyquin induction of Prx I coincided with an increase in p38 MAPK activation that was nearly eliminated by siRNA knock-down of Prx I expression. Finally, the Prx I-regulated activation of p38 MAPK was studied under the conditions of macrophage foam cell formation. In these experiments, exposure to oxLDL resulted in both the dose-dependent upregulation of Prx I and the activation p38 MAPK. Prx I siRNA decreased the oxLDL-induced upregulation of Prx I and led to a highly attenuated, but dose-dependent activation of p38 MAPK. When treated with 50 $\mu$g/mL oxLDL, activation of p38 MAPK was greater in control macrophages compared to Prx I-KD macrophages. However, the decrease in Prx I expression from control to Prx I-KD macrophages was much larger than the corresponding decrease in p38 MAPK activation. This observation contrasts with the ethoxyquin-induced activation of p38 MAPK, where negligible activation was observed in Prx1-KD macrophages. These data suggest that other factors may be induced during foam cell formation that contribute to p38 MAPK activation, and are not induced by ethoxyquin-treatment. A potential mechanism may exist where partial p38 MAPK activation occurs when Prx I expression is limited. As a group, however, these data demonstrate that p38 MAPK activation is largely dependent on Prx I.

Activation of p38 MAPK has been linked to the downstream initiation of Prx I gene expression in macrophages (77), as well as the posttranscriptional regulation of Prx I expression in osteoblasts (78). Accordingly, a feed-forward mechanism may exist in which Prx I maintains p38 activity, resulting in a continued increase in Prx I expression and a sustained p38 MAPK response. One could speculate that the effect of this response would be directed toward the initiation of apoptosis, considering the several pro-apoptotic transcription factors that are targets of p38 MAPK phosphorylation. The observation that the pro-survival Akt pathway was not activated following treatment with ethoxyquin or oxLDL, nor did the inhibition of Prx I have an effect on Akt activation, would support this scenario.

In summary, our data support a functional model in which Prx I has at least two distinct roles — as an antioxidant enzyme and as a regulator of p38 MAPK. The antioxidant functionality of Prx I was demonstrated in this work to be cytoprotective and not dependent on the p38 MAPK activation. However, the antioxidant activity of Prx I may not be as much pro-survival as it is anti-necrosis. One could speculate that the antioxidant role of Prx I helps to maintain macrophage foam cells in the atherosclerotic lesion by preventing an undesirable necrotic cell death. At the same time, Prx I also maintains the activation of p38 MAPK, providing the option for the downstream initiation of apoptosis. This concept overlaps with observations made for cancerous cells in tumors, where Prx I upregulation is well-documented. In both cancerous cells and foam cells, the cells have transformed to a pathological state where an apoptotic death may be a more beneficial result. Prolonged tumor cell survival leads to tumor growth and metastasis.
Similarly, prolonged foam cell survival is pro-atherogenic by promoting a continued inflammatory response with its downstream effects. Further study of Prx I expression and function will continue to resolve the roles of this enzyme in these downstream effects.

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ABBREVIATIONS

Abbreviations used are as follows: Prx, peroxiredoxin; LDL, low-density lipoprotein; oxLDL, oxidized low-density lipoprotein; siRNA, small interfering ribonucleic acid; ROS, reactive oxygen species; CM-H$_2$DCFDA, 5-(and-6)-chloromethyl-2'7'-dichlorodihydrofluorescein diacetate acetyl ester; MAPK, mitogen-activated protein kinase; t-BOOH, tert-butyl hydroperoxide; PTEN, phosphatase and tensin homolog; PIP3, phosphatidylinositol 3,4,5-trisphosphate; TNF-α, tumor necrosis factor alpha; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; TNBS, trinitrobenzene sulfonate; DMEM, Dulbecco's Modified Eagle Medium; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HRP, horseradish peroxidase; IPG, immobilized pH gradient; PVDF, polyvinylidene fluoride.
FIGURE LEGENDS

Fig. 1. Identification of Prx I as a protein upregulated during macrophage foam cell formation. J774 murine macrophages were lysed in 1% SDS buffer and assayed for protein concentration. 1 mg total protein was acetone-precipitated, then prepared for 2D gel separation. A, 2D SDS-PAGE gel (12.5% Tris-HCL) showing the entire pI range 3 – 10 and molecular weights of approximately 10 – 100 kDa. A band identified by LC-ESI mass spectrometry as Prx I is labeled. B, Magnified inset from 2D gel showing Prx I in control (untreated) macrophages, compared to C, macrophages treated with 50 μg/mL oxLDL for 24 hours.

Fig. 2. Western blot analysis of the time- and dose-dependent upregulation of Prx I following exposure to oxLDL. A, J774 murine macrophages were left untreated (Ctl), or treated with 50 μg/mL LDL or oxLDL for 24 and 48 hours. B, Macrophages were treated with 0, 10, 25 or 50 μg/mL oxLDL for 24 hours. In both A and B, protein was detected using a rabbit polyclonal antibody to Prx I (upper panel) or mouse monoclonal antibody to GAPDH as a loading control (lower panel).

Fig. 3. Detecting the oxidative inactivation of Prx I by 2D SDS-PAGE Western blot analysis. J774 murine macrophages were left untreated (Ctl) (A & D), treated with 100 μM t-BOOH (B & E) or 50 μg/mL oxLDL (C & F) for 24 hours. After oxidant-exposure, macrophages were collected and prepared for 2D SDS-PAGE separation. 2D gels were transferred to PVDF, and Western blot analysis was carried out using antibodies for Prx I (A, B, C) or oxidatively-modified Prx I (oxPrx I) (D, E, F). The three protein bands seen in each panel correspond to, from left to right, inactive Prx I (Cys-SO3 modification), inactive Prx I (Cys-SO2 modification), and active Prx I (unoxidized). The oxPrx I antibody is designed to detect both Cys-SO3 and Cys-SO2 modifications.

Fig. 4. Effects of ethoxyquin treatment on Prx I induction. A, Ethoxyquin-induced upregulation of Prx I. J774 murine macrophages were exposed to 0 – 100 μM ethoxyquin for 24 hours, then collected and analyzed by Western blot. B, Effect of ethoxyquin pre-treatment in the oxLDL-induced upregulation of Prx I. Macrophages were treated with 50 μM ethoxyquin or left untreated for 24 hours, then treated with 0, 25 or 50 μg/mL oxLDL for 24 hours. In A and B, panels depict Western blots using antibodies for Prx I (upper panels) or GAPDH as a loading control (lower panels).

Fig. 5. Knock-down of Prx I expression by siRNA transfection. A, J774 macrophages were transfected for 24 hours with varied concentrations of siRNA targeting exon 4 of Prx I mRNA. B, Cells were not transfected (Ctl), exposed to transfection vehicle only (Veh), or transfected with scrambled (Scr), reverse (Rev), or Prx I sequence siRNA (Prx I). C, Cells were transfected with reverse sequence (Rev) or Prx I siRNA (Prx I), then exposed to 0 or 50 μg/mL oxLDL for 24 hours. In A, B, and C, panels depict Western blots using antibodies to Prx I (upper panels), Prx II (middle panels), and GAPDH (bottom panels).

Fig. 6. Oxidant-induced cytotoxicity in macrophages with induced or knocked-down expression of Prx I. J774 murine macrophages were treated with A, 0 – 200 μM t-BOOH or B, 0 – 100 μg/mL oxLDL for 48 hours, and assayed for the presence of respiring cells. Results are expressed as % cell survival normalized to untreated controls. The data are labeled as follows: (♦) Ctl: macrophages transfected with reverse-sequence siRNA, (◊) Prx I-KD: macrophages transfected with Prx I siRNA for 24 hours, (■) + Ethx: macrophages transfected with reverse-sequence siRNA, then treated with 50 μM ethoxyquin for 24 hours prior to oxidant-exposure, (□) Prx I-KD, + Ethx: macrophages transfected with Prx I siRNA for 24
hours, then exposed to 50 μM ethoxyquin for 24 hours. Results represent an average of three independent treatments. Error bars represent ± one standard deviation.

**Fig. 7.** Oxidant-induced ROS generation in macrophages with induced or knocked-down expression of Prx I. J774 murine macrophages were treated with A, 0, 25 or 50 μM t-BOOH or B, 0, 10 or 50 μg/mL oxLDL for 24 hours, and assayed for the generation of ROS. Results are expressed as relative ROS normalized to untreated Ctl macrophages. The data are labeled as follows: (♦) Ctl: macrophages transfected with reverse-sequence siRNA, (◊) Prx I-KD: macrophages transfected with Prx I siRNA for 24 hours, (■) + Ethx: macrophages transfected with reverse-sequence siRNA, then treated with 50 μM ethoxyquin for 24 hours prior to oxidant-exposure, (□) Prx I-KD, + Ethx: macrophages transfected with Prx I siRNA for 24 hours, then exposed to 50 μM ethoxyquin for 24 hours. Results represent an average of three independent treatments. Error bars represent ± one standard deviation.

**Fig 8.** Prx I regulates the activation of p38 MAPK stimulated by H2O2, ethoxyquin, and oxLDL. A, H2O2-stimulated p38 MAPK activation. Ctl (transfected with reverse-sequence siRNA) and Prx I-KD macrophages were exposed to H2O2 for the indicated times, collected, and prepared for Western blot analysis. The knocked-down expression of Prx I is demonstrated by Western blot of Prx I (upper panel). Activation of p38 MAPK by dual-phosphorylation (middle panel) and total p38 MAPK (bottom panel) were also determined by Western blot analysis. B, The effect of ethoxyquin-induced Prx I overexpression on p38 MAPK activation. Ctl and Prx I-KD macrophages were exposed to 50 μM ethoxyquin for 24 hours, or left untreated. Macrophages were collected and analyzed by Western blot for Prx I (top panel), phosphorylated p38 MAPK (2nd from top), total p38 MAPK (3rd from top), Akt activated by phosphorylation at Ser-473 (2nd from bottom) and total Akt (bottom panel). C, oxLDL-stimulated p38 MAPK activation. Ctl and Prx I-KD macrophages were treated with 0, 10, 25 or 50 μg/mL oxLDL for 24 hours, collected and analyzed by Western blot. Panels are identical to the listing in B.

**Fig 9.** Reduction of ROS in macrophages with inhibited p38 MAPK activity. Macrophages were treated with 50 μM ethoxyquin for 24 hours to induce Prx I expression, or left untreated. The p38 MAPK inhibitor SB-203580 was added to half of the ethoxyquin-treated and untreated samples. All samples were exposed to 200 μM t-BOOH for 2 hours and ROS levels were assayed by flow cytometry. (♦) Control macrophages, (□) macrophages with p38 MAPK inhibitor added. Results represent an average of three independent experiments. Error bars represent ± one standard deviation. * p < 0.05 compared to the corresponding value without ethoxyquin treatment (0 μM).
Figure 2
Figure 3

A. Ctrl
B. t-BOOH
C. oxLDL
D. Prx I
E. Cys-SO₃
F. Cys-SO₂

Active Prx I
Figure 4

Panel A:
- Prx I
- GAPDH
- ethx (μM): 0 10 25 50 75 100

Panel B:
- Prx I
- GAPDH
- oxLDL (μg/mL): 0 25 50
  - no pre-treatment
  - ethoxyquin pre-treatment
Figure 5
Figure 7

A. Relative ROS vs. t-BOOH (μM)

- Ctl
- + Ethx
- Prx I-KD
- Prx I-KD, + Ethx

B. Relative ROS vs. oxLDL (μg/mL)

- Ctl
- + Ethx
- Prx I-KD
- Prx I-KD, + Ethx
Figure 8

A. H$_2$O$_2$ exposure (min):

B. ethx (μM): 0 50 0 50

C. oxLDL (μg/mL): 0 10 25 50 0 10 25 50

--- Ctl ---

--- Prx I-KD ---

Prx I
phos-p38
total p38
Figure 9

The graph illustrates the effect of Ethoxyquin on ROS levels in control and p38 MAPK inhibited cells. The x-axis represents Ethoxyquin concentration in μM, while the y-axis shows Relative ROS. The graph shows a decrease in ROS levels with increasing Ethoxyquin concentration, indicating a potential inhibition of ROS production. The asterisks denote significant differences between the control and treated groups.
Dual role of peroxiredoxin I in macrophage-derived foam cells
James P. Conway and Michael Kinter

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