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Oxidative Stress-inducible Proteins in Macrophages

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Macrophages produce reactive oxygen species such as O_2^-, H_2O_2 and $^{\bullet}OH$ that contribute to the pathogenesis of diseases such as inflammation and atherosclerosis. The cells have multiple defense systems against those reactive oxygen species, and we describe here such an oxidative stress-inducible defense system. Upon exposure to reactive oxygen species and electrophilic agents, murine peritoneal macrophages induce stress proteins to protect themselves. Using differential screening, we cloned two novel proteins designated MSP23 and A170 that are induced in the cells by low levels of reactive oxygen species, electrophilic agents and other oxidative stress agents. MSP23 is murine peroxiredoxin I having a thioredoxin peroxidase activity and A170 is known as an ubiquitin- and PKC ξ-binding protein. In addition to these two proteins, heme oxygenase-1 (HO-1) and cystine transport activity are also induced in the cells under oxidative stress conditions. Using *nrf2*-deficient macrophages, we found that transcription factor Nrf2, which is known to interact with antioxidant responsive elements (AREs) in the regulatory sequences of the genes, plays an important role in the oxidative stress-inducible response in the cells.

Keywords: Macrophage, HO-1, peroxiredoxin, diethylmaleate, A170

INTRODUCTION

In this paper we focus on an inducible adaptation system of murine peritoneal macrophages against reactive oxygen species (ROS), electrophilic agents and other oxidative stress agents. We have characterized diethylmaleate (DEM)-inducible genes in murine peritoneal macrophages to study the adaptive response to oxidative stress. Diethylmaleate is an electrophilic agent reactive with sulfhydryl compounds and known as a GSH depleter. One of the important activities induced by diethylmaleate is a sodium-independent cystine transport activity termed system x_c^-.[1] The uptake of cystine across the plasma membrane is the rate-limiting step to maintain synthesis of GSH when cells are placed under air-exposed culture conditions.[2] The expression level of the cystine transport activity is quite low in freshly prepared cells, and is markedly induced during culture in a medium supplemented with stress agents.[3]

We monitored DEM-inducible protein synthesis by metabolic labeling with [^{35}S]Met and

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observed 32 and 23 kDa[4] proteins by fluorography. The former was identified as HO-1[5] and the latter was cloned and termed MSP23.[6] We then characterized more systematically the DEM-inducible cellular response by isolating DEM-inducible cDNAs using differential screening. The cDNA library was constructed using an mRNA fraction prepared from DEM-treated cells, and both positive and negative cDNA probes were used to select differentially expressed clones.[5] We isolated many cDNA clones by this method and analyzed the encoded proteins. In summary, we found two novel stress-inducible proteins among the selected clones (Table I). We termed the first protein as MSP23,[6] the 23 kDa macrophage stress protein. The second novel protein is A170,[7] which we tentatively refer to by clone number. Treatment of the macrophages with 0.05–1.0 mM DEM induced both MSP23 and A170 mRNAs in a dose-dependent manner (Figure 1, lanes 1–5). Other electrophilic agents, catechol and 1-chloro-2,4-dinitrobenzene

TABLE I Oxidative stress-inducible proteins in murine macrophages

Proteins	Apparent mass (kD) in SDS-PAGE	Characteristics
MSP23 (peroxiredoxin I)[6]	23	Thioredoxin peroxidase[11] Heme-binding[14]
A170[7]	60	PKC ξ-binding (ZIP)[18] Ubiquitin-binding (p62)[20]
Heme oxygenase-1[5]	32	Decomposition of heme

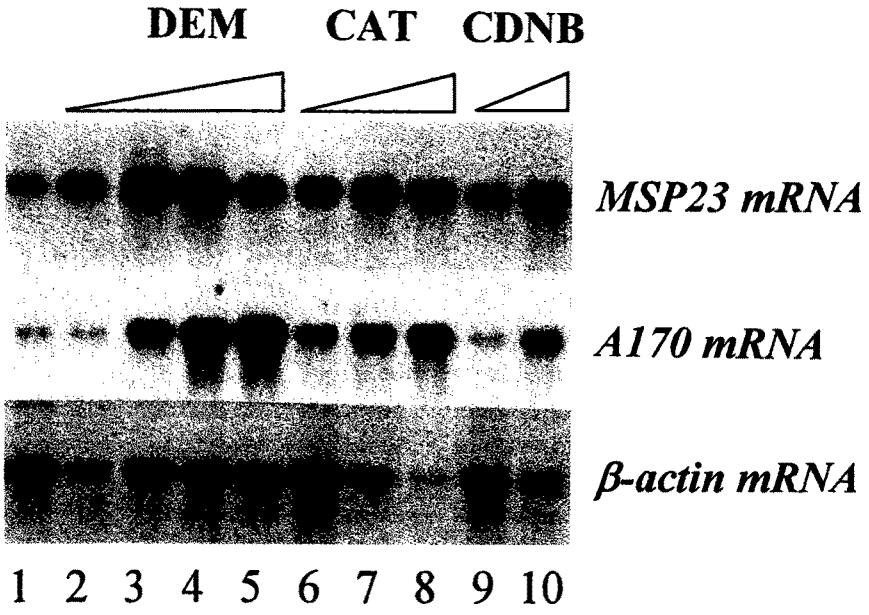

FIGURE 1 Induction of MSP23 and A170 mRNAs in murine macrophages by electrophilic agents. Each agent was added to the medium after 1 h of plating macrophages and cultured for an additional 6.5 h before extracting RNA. Total RNA (1.5 μg/well) was used for Northern blotting as described previously.[7] Lane 1, control; lane 2, +50 μM DEM; lane 3, +200 μM DEM; lane 4, +500 μM DEM; lane 5, +1 mM DEM; lane 6, +8 μM catechol; lane 7, +40 μM catechol; lane 8, +80 μM catechol; lane 9, +1 μM CDNB; lane 10, +5 μM CDNB. The levels of β-actin mRNA apparently decreased during the treatment of the macrophages with high concentrations of catechol and CDNB.

(CDNB), also induced both mRNAs in the cells (Figure 1, lanes 6–10). In addition to these two clones, we also selected many cDNA clones encoding HO-1,[8] well-known stress-inducible protein that degrades heme to CO, iron and biliverdin. We were unsuccessful in finding a clone encoding the cystine transporter among those selected by a functional assay.

CHARACTERIZATION OF MSP23 AND A170 PROTEINS

MSP23 belongs to a new type of antioxidative protein family named peroxiredoxin (Prx). It is markedly induced in the macrophages by diethylmaleate, hydrogen peroxide, sodium arsenite and cadmium chloride.[6] Prx has one or two conserved cysteine residue(s) which react with radicals[9] and peroxides.[10,11] The Prx of eukaryotes has a thioredoxin peroxidase activity and efficiently reduces hydrogen peroxide in the presence of thioredoxin/thioredoxin reductase/NADPH.[11] This new peroxidase system is independent from the GSH-peroxidase system and seems to be important to protect cells under GSH depletion or with low GSH-peroxidase activity. In addition to MSP23, Prx I class includes PAG (proliferation associated gene; human),[12] NKEF A (natural killer enhancing factor; human)[13] and HBP23 (heme-binding protein; rat).[14]

The yeast peroxiredoxin was initially called a thiol-specific antioxidant, TSA, that protected the activity of glutamine synthetase from oxidative damage.[15] MSP23 is expressed in many tissues and the expressed level is especially high in liver and in kidney. Using purified MSP23 from liver, we confirmed that it exhibits TSA activity.[16] We then examined the effect of hemin on TSA activity since MSP23 has a high affinity to heme.[14] We found that a few micromolars of hemin inhibited the antioxidative activity of MSP23.[16] Therefore induction of HO-1, which removes heme, may be important to protect the antioxidative activity of peroxiredoxin.

A170 protein is a new class of stress-inducible protein having no structural homology to other antioxidative proteins.[7] This protein of 442 amino acid residues has a Zn-finger motif, a PEST motif (a signal for rapid degradation) and several potential phosphorylation sites for serine/threonine kinases. Caseine kinase II, PKA and ERK 2 respectively phosphorylated this protein *in vitro*.[17] It has an apparent molecular mass of 60 kDa in SDS-PAGE, although the calculated mass from the amino acid composition is about 48 kDa. A170 is rich in proline and glutamate residues which may be the cause of this abnormal mobility. Paraquat, cadmium chloride and diethylmaleate enhanced the level of the transcript in the cells.[7] The rat homologue of A170 was isolated as a PKC ξ-binding protein.[18] Interestingly, p62, a 62 kDa human lymphocyte protein 90% identical to A170, has been cloned and characterized as a phosphotyrosine-independent ligand of the SH2 domain of tyrosine kinase $p56^{lck}$.[19] The C-terminal domain of p62, which is completely conserved in A170, has an affinity to ubiquitin.[20] We found that proteasome inhibitors induced A170.[21] These results suggest that the A170 protein plays a role in the metabolism of ubiquitinated protein under oxidative stress.

TRANSCRIPTIONAL REGULATION OF THE STRESS PROTEINS

Table II summarizes stress agents that commonly induce mRNAs of HO-1, MSP23 and A170. All of

TABLE II Common inducers of the HO-1, MSP23 and A170 mRNAs in macrophages[24]

1. Electrophilic agents
diethylmaleate,
catechol,
1-chloro-2,4-dinitrobenzene
2. Reactive oxygen species
glucose oxidase/glucose (H_2O_2),
paraquat, menadione,
t-butylhydroquinone
3. Others
sodium arsenite,
cadmium chloride

these stress agents have a reactivity to sulfhydryl residues. Recently, a transcription factor Nrf2 has been shown to regulate the electrophile-induced expression of the phase II detoxifying enzymes via antioxidant response elements.[22] The induction of the phase II enzymes in intestine, liver and other tissues by electrophiles is called "electrophile counterattack response".[23] Animals developed this defense system against toxic electrophilic compounds by conjugating them with GSH to excrete them from the body. We found that the electrophile-inducible system in macrophages is also regulated by Nrf2. Using macrophages from the *nrf2*-knockout mice, we have shown that Nrf2 is the major transcriptional factor that regulates the induction of HO-1, MSP23, A170 and the cystine transport activity by electrophiles and ROS.[24] *nrf2*-deficient macrophages have severe defects in the induction of stress proteins and were much more sensitive to oxidative stress than *nrf2*-heterozygous or wild type macrophages.[24] Thus the induction of these stress proteins by Nrf2 plays an important role in the defense reaction against a wide range of electrophilic compounds and ROS.

CONCLUSION

In summary, exposing thioglycollate-elicited macrophages to electrophilic agents or ROS induces cystine transport activity in the plasma membrane, HO-1 in microsomes to degrade heme, MSP23 in cytosol to reduce peroxides, and A170 in cytosol to interact with ubiquitinated proteins.[24] These induced proteins protect cells from damage by electrophiles and ROS, and probably also by heavy metals. This response is mainly regulated by transcription factor Nrf2 that interacts with AREs in the regulatory region of the genes.[23] The activation mechanism of transcription factor Nrf2[25] is entirely different from previously known redox-regulated transcription factors, NF-κB and AP-1. The latter two factors activate genes involved in cell activation, differentiation and transformation, rather than cell protection. Therefore, Nrf2 is suggested to be the main factor activated by electrophiles and ROS to induce the expression of genes that work for cell protection.

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