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Original Contribution

Glutathione depletion inhibits lipopolysaccharide-induced intercellular adhesion molecule 1 synthesis

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Abstract

Cellular redox status is known to regulate a number of biological processes, including the activation of inflammatory genes. Our previous studies demonstrated that thiol depletion using diethyl maleate (DEM) reduced neutrophil sequestration in animal models of inflammation, an effect primarily mediated by impaired upregulation of the adhesion molecule, ICAM-1. The present studies were performed to discern the mechanism whereby DEM prevents LPS-induced ICAM-1 expression in human umbilical vein endothelial cells. DEM caused a time- and concentration-dependent inhibition of ICAM-1 expression in LPS-stimulated HUVEC by blocking induction of gene transcription. Interestingly, DEM had little effect on the degradation of the inhibitory protein IκB-α, but rather appeared to prevent translocation of the transcription factor NF-κB into the nucleus. Readdition of glutathione following DEM treatment restored the ability of LPS to induce NF-κB translocation and ICAM-1 synthesis. DEM plus LPS caused synergistic induction of heme oxygenase-1 (HO-1), suggesting its role in the inhibitory effects of DEM. However, HO-1 was shown to be neither sufficient nor necessary for the anti-inflammatory effects of glutathione depletion. These studies illustrate that thiol depletion may represent a potential therapy for inflammation, exerting its effects via a distinct mechanism on cell signaling pathways.
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Keywords: Glutathione depletion; Diethyl maleate; Heme oxygenase; HUVEC; Lipopolysaccharide; ICAM-1; Free radicals

Introduction

Organ dysfunction is one of the hallmarks of the excessive inflammatory response in the critically ill septic patient and correlates with poor outcome in this population. While a number of mechanisms are known to be involved in the development of this state, oxidative stress seems to be a major contributor. Oxidative stress per se is not only capable of directly causing cellular damage, but accumulating evidence also suggests that alterations in the redox state can trigger or modify a variety of biological processes (reviewed in [1,2]). Among these, the redox status of the cells is known to have an impact on the expression of inflammatory genes, an effect which may further exacerbate tissue injury [1–3]. A central theme in early signaling initiated by inflammatory stimuli is the activation and subsequent nuclear translocation of transcription factors belonging to the nuclear factor kappa B (NF-κB) family (reviewed in [4]). Binding of these heterodimers to the promoter region of a number of proinflammatory genes is critical to induction of their transcription. Oxidative stress that often associates with inflammatory conditions can

Abbreviations: CrMP, chromium mesoporphyrin; DEM, diethyl maleate; DTT, dithiothreitol; EMSA, electrophoretic mobility-shift assay; GSH, glutathione; GSH-MEE, glutathione monoethyl ester; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NPSH, nonprotein sulfhydryls; NF-κB, nuclear factor kappa B; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; TBS, Tris-buffered saline; TNB, 5-thio-2-nitrobenzoic acid.

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activate a number of signaling pathways and induce transcription of inflammatory genes [5,6]. The importance of the redox status of the cell has prompted several laboratories, including our own, to investigate the effects of redox manipulation on cellular function, both in vitro and in vivo, as a means of understanding its physiological significance as well as developing new therapeutic strategies. Alterations in the redox balance in various cell types have been shown to influence NF-κB translocation and activation by several studies [7]. For example, augmentation of intracellular antioxidant capacity using *N*-acetylcysteine has been shown to inhibit activation of inflammatory cells in vitro as well as lipopolysaccharide (LPS)-induced organ injury in vivo [8,9]. One recent report demonstrated that antioxidants inhibited LPS-induced NF-κB translocation in neutrophils through effects on proximal signaling molecules such as IRAK-1 and IRAK-4 in the LPS-NF-κB pathway [10]. Paradoxically, our group has found that intracellular thiol depletion by the glutathione-depleting agent diethyl maleate (DEM) was able to prevent LPS-induced lung and liver injury [8,11]. Impaired upregulation of endothelial cell adhesion molecules was thought to contribute to the ability of DEM to minimize organ neutrophil sequestration and injury. In vitro, GSH depletion was also shown to inhibit expression of the intercellular adhesion molecule-1 (ICAM-1) on the surface of human umbilical vein endothelial cells (HUVEC) and thereby prevent neutrophil adhesion and transmigration [8]. However, while the anti-inflammatory effects of the antioxidants such as *N*-acetylcysteine have been correlated with their ability to inhibit upstream LPS signaling pathways which culminate in NF-κB translocation, the mechanisms whereby thiol-oxidizing agents exert their effects on adhesion molecule expression have not been elucidated.

The present studies were performed to investigate the cellular mechanisms whereby thiol glutathione depletion is able to impair expression of ICAM-1 in HUVEC following treatment with LPS. The studies demonstrate that DEM is able to induce a time- and dose-dependent inhibition of LPS-induced ICAM-1 mRNA expression, through its ability to inhibit NF-κB nuclear translocation and subsequent ICAM-1 gene transcription. The inhibitory effect was reversed by reestablishing the level of intracellular glutathione, consistent with the conclusion that glutathione depletion contributed to the effect. Interestingly, the major action of DEM was not through an effect on upstream signaling pathways leading to degradation of the inhibitory protein IκB-α, an event generally required for nuclear translocation of NF-κB. Rather, DEM treatment appeared to exert its inhibition by precluding translocation of liberated NF-κB into the nucleus. The combination of DEM plus LPS also caused synergistic induction of heme oxygenase-1 (HO-1), suggesting a mechanism for the inhibitory effect of DEM. However, HO-1 expression proved to be neither necessary nor sufficient to explain the inhibition by DEM.

Materials and methods

Reagents

Escherichia coli 0111:B4 lipopolysaccharide, diethyl maleate, L-buthionine-sulfoximine, hemin, and bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, MO). Phorone was purchased from Fluka Chemical Corp. (Oakville, ON). Glutathione monoethyl ester (GSH-MEE) was from Calbiochem. Endothelial cell growth supplement was purchased from Biomedical Technologies Inc. (Stoughton, MA). The following antibodies were used: goat polyclonal anti-p65, monoclonal anti-ICAM-1, rabbit polyclonal anti-VCAM-1, and anti-IκB-α of human origin from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); and monoclonal anti-heme oxygenase-1 antibody from Stressgene. Horseradish peroxidase-conjugated secondary antibodies were from Amersham. The enhanced chemiluminescence kit was purchased from Perkin-Elmer. Human ICAM-1 cDNA probe was a kind gift from Dr. T. Springer (Boston, MA). Human heme oxygenase-1 cDNA probe was a kind gift from Dr. P. Marsden (Toronto, ON). The heme oxygenase inhibitor chromium mesoporphyrin (CrMP) was from Frontier Scientific, Inc. (Logan, UT).

Isolation and culture of human umbilical vein endothelial cells

Human umbilical vein endothelial cells were isolated and subcultured as previously described [12]. Briefly, umbilical cord veins were cannulated and flushed with copious volumes of sterile normal saline. Ten milliliters of 0.2% Collagenase Type IV (Worthington Biochemical, Freehold, NJ) was infused and incubated at 37°C for 15 min. Cords were flushed with 30 ml PBS, and the effluent was collected in sterile tubes. Cells were sedimented and resuspended in 10 ml culture medium (80% medium 199, 20% fetal calf serum supplemented with 2 mM L-glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, 50 μg/ml endothelial growth supplement, and 100 μg/ml heparin). Cells were grown in tissue culture-treated flasks and plates (Corning). For immunofluorescence, cells were plated on coverslips coated with 2% gelatin (Difco media) for 10 min at room temperature.

Treatment of human umbilical vein endothelial cells

HUVEC were pretreated with DEM (50–500 μM) for 30 min or the heme oxygenase inhibitor CrMP (1 h) followed by stimulation with 1 μM LPS for the indicated time in the presence of 10% fetal calf serum. To restore glutathione levels following DEM treatment cells were pretreated with 250 μM DEM for 30 min. The drug was then washed away and cells were incubated with 10 mM glutathione monoethyl ester prior to treatment with LPS for the indicated time.

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