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Effect of Rhinovirus Challenge on Antioxidant Enzymes in Respiratory Epithelial Cells

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The host inflammatory response appears to be an important contributor to the pathogenesis of human viral respiratory illness. Virus-induced oxidative stress appears to mediate an early phase of elaboration of the proinflammatory cytokine interleukin-8 by respiratory epithelial cells. The purpose of these studies was to determine if virus-induced alterations in either the expression or function of antioxidant enzymes contributes to the cellular oxidative stress following rhinovirus challenge. The activities of Mn superoxide dismutase (MnSOD), catalase and glutathione peroxidase (GPX) were not significantly changed by rhinovirus challenge. CuZn superoxide dismutase (CuZnSOD) activity six hours after challenge was 2.55 ± 0.56 U/mg protein in rhinovirus-challenged cells compared to 1.16 ± 0.54 U/mg protein in control cells ($p = 0.029$). This increased activity was associated with a concomitant increase in CuZnSOD mRNA and protein concentration. These data suggest that rhinovirus-induced changes in the host cell redox state that result in the early elaboration of interleukin-8 are not mediated by inhibition of either the expression or function of these antioxidant enzymes.

Keywords: Antioxidant; Rhinovirus; Common cold; Interleukin-8

INTRODUCTION

The host inflammatory response appears to play an important role in the pathogenesis of viral respiratory disease (reviewed in Ref. [1]). A consistent feature of the host response has been elaboration of interleukin-8 by respiratory epithelial cells with subsequent recruitment of polymorphonuclear leukocytes to the nasal mucosa. During infection with rhinovirus, the most frequent cause of the common cold, there is correlation between both interleukin-8 and polymorphonuclear leukocyte concentrations in the nasal secretions and the presence or severity of common cold symptoms.[2] An understanding of the mechanism of cytokine expression in response to virus challenge may suggest new potential targets for intervention in these illnesses.

The elaboration of IL-8 by respiratory epithelial cells following virus challenge appears to follow a biphasic pattern.[3,4] The late phase of IL-8 expression occurs approximately 24 h after virus challenge, requires virus replication and involves activation of p38 kinase.[4] In contrast, the early phase of IL-8 expression occurs within 2–6 h after virus challenge and is not dependent upon virus replication[3,5] or activation of mitogen-activated protein kinase pathways (authors' unpublished observations). A role for oxidant stress in the early phase response to virus challenge is suggested by the demonstration of oxidant stress in rhinovirus challenged cells and fact that interference with the oxidant stress response by pretreatment of the cells with an antioxidant prevents the early phase elaboration of IL-8.[5]

Cellular oxidative stress is the result of a disturbance of the prooxidant and antioxidant balance in the cell. Eukaryotes have evolved several different antioxidant enzymes to detoxify reactive oxygen species. The detoxification of superoxide via the dismutation reaction is catalyzed by two structurally distinct enzymes. CuZn superoxide dismutase (CuZnSOD) is a constitutively expressed cytoplasmic enzyme while Mn superoxide dismutase

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(MnSOD) is located primarily in mitochondria and is induced by oxidative stress as well as cytokines.[6–9] H_2O_2 produced by the dismutation reaction is catabolized to water by catalase in peroxisomes and by glutathione peroxidase (GPX) in cytoplasm.[10] While alteration of either the expression or the function of these antioxidant enzymes by rhinovirus infection might be expected to contribute to cellular oxidative stress, the effect of rhinovirus infection on cellular antioxidant enzymes has not been reported. The purpose of this study was to determine whether rhinovirus infection alters either the expression or function of cellular antioxidant enzymes in a manner that would contribute to virus-induced oxidative stress and subsequent IL-8 elaboration.

MATERIALS AND METHODS

Materials

Rabbit anti-rat CuZnSOD and MnSOD antisera, and CuZnSOD and MnSOD cDNA probes were kindly provided by Dr K. Asayama, Yamanashi Medical University, Japan. Ultraspec-II RNA solution was from Biotecx (Houston, TX). Recombinant human IL-8 was from R&D System Inc. (Minneapolis, MN). All other chemicals used in the study were from Sigma Chemical Company (St. Louis, MO).

Cell Culture

Transformed human bronchial epithelial cells (BEAS-2b; American Type Culture Collection, Rockville, MD) were grown in bronchial epithelial growth medium (BEGM; Clonetics; Minneapolis, MN) supplemented with human recombinant epithelial growth factor (0.5 ng/ml), insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), epinephrine (0.5 μg/ml), transferrin (10 μg/ml), gentamicin (50 μg/ml) and amphotericin B (50 ng/ml). Cells were used for experiments at passages 40–55, when the monolayers were 75–80% confluent.

Viral Preparation and Infection

Rhinovirus, type 39 (RV39) was grown in HeLa-I cells (provided by F.G. Hayden, University of Virginia HSC, Charlottesville, VA). HeLa-I cells infected with RV39 were mechanically collected, lysed by freeze-thaw and clarified via centrifugation at 2000*g* (Beckman GPR centrifuge, Beckman Instruments, Inc., Palo Alto, CA). For all experiments, BEAS-2b cells grown in T-75 flasks were challenged with RV39 at 100 $TCID_{50}$/cell in a final volume of 12 ml/flask and incubated at 33°C for 1 h, cells were then washed three times with media and further incubated with fresh media for 4, 6 or 24 h.

Measurement of Antioxidant Enzyme Activities

At the specified times after virus challenge, cells were collected and washed three times with PBS and sonicated for 3–5 min, the samples were then subjected to total protein and enzyme assays as follows. Total protein was estimated by the method of Bradford.[11] Total SOD, MnSOD and CuZnSOD activities were determined by a previously described method.[12] Catalase activity was measured according to the method of Baudhuin *et al.*[13] and GPX activity was measured by the enzyme coupled method of Beutler *et al.*[14] as modified by Asayama.[15]

Immunoblot Analysis of the CuZn SOD and MnSOD Proteins

Equal amounts of cell homogenate proteins were electrophoretically resolved on a 15% slab gel. After electrophoresis, the proteins were transferred onto a polyvinylidine difluoride membrane. They were incubated at 4°C with primary antibodies followed by the incubation with horseradish peroxidase-conjugated IgG for 30 min at room temperature. Each immune complex was visualized by diaminobenzidine reaction.

Northern Blot Analysis for SODs

Total RNA was isolated from control and RV-39 infected BEAS-2b cells using Ultraspec-II RNA isolation system (Biotecx, Houston, Tx). Aliquots containing 15 μg RNA from each sample were electrophoretically resolved on a 1.2% denaturing formaldehyde–agarose gel, transferred to nylon membrane and cross linked using UV Stratalinker (Stratagene, La Jolla, CA). Radioactive DNA probes were prepared according to the instruction provided with Ready-To-Go DNA labeling kit (Amersham Pharmacia Biotech, Inc. Piscataway, NJ). Northern blot analysis was performed essentially as described for Express Hybridization Solution (Clontech, Palo Alto, CA). CuZn SOD and MnSOD cDNAs from rat were used as probes.[16] Hybridization with rat and human cDNA have been reported to produce identical results.[17] Glyceraldehyde-3 phosphate dehydrogenase cDNA probe was used as standard for comparing hybridization signals. Northern blots were scanned by the Bio-Rad Imaging densitometer (Model GS-670) and densities were quantified by using image analysis software, Molecular Analyst PC (Bio-Rad, Hercules, CA).

Statistical Analysis

Where appropriate, the statistical significance of results was determined by nonparametric testing with the Mann–Whitney U test. All data are reported

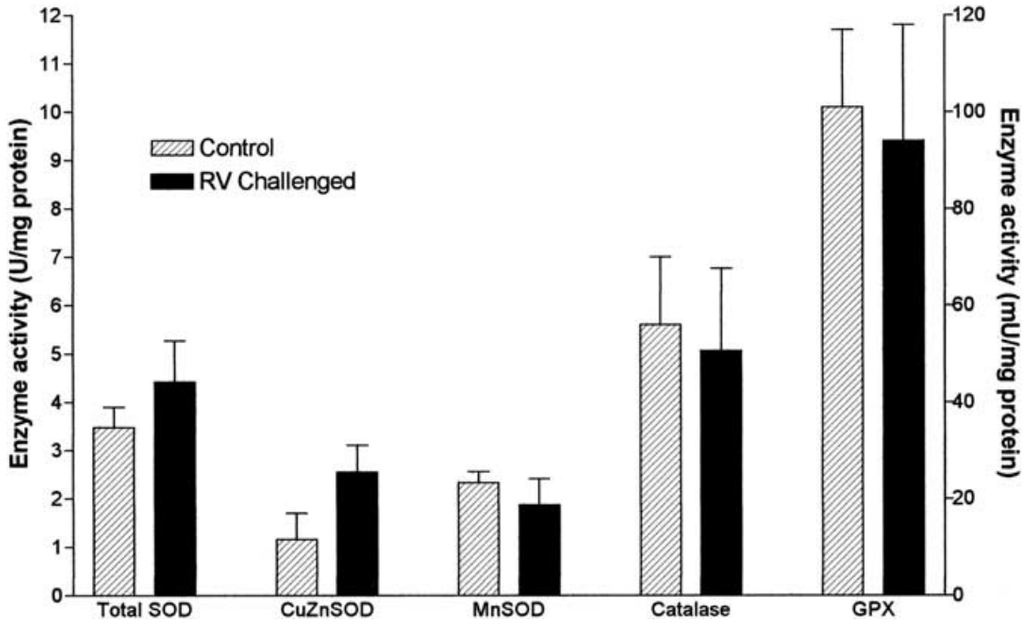

FIGURE 1 Activities of antioxidant enzymes in BEAS-2b cells with and without rhinovirus challenge. The CuZnSOD activity is significantly higher (0.029, Mann–Whitney U test) in virus challenged cells. The activity of glutathione peroxidase (GPX) is expressed as mU/mg protein (right axis).

as the mean ± standard deviation (SD) of at least three triplicate experiments.

RESULTS

Effect of Rhinovirus Challenge on Antioxidant Enzyme Activity

Six hours after challenge, the specific activity of CuZnSOD was found to be significantly higher ($p = 0.029$) in RV challenged cells compared to unchallenged controls. In contrast, there were no significant changes observed in total SOD or MnSOD activity (Fig. 1). Rhinovirus challenge had no effect on the specific activities of the two H_2O_2 degrading enzymes, catalase and GPX (Fig. 1).

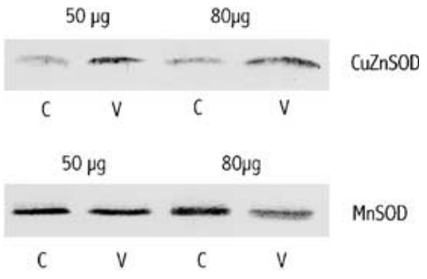

FIGURE 2 Representative western blot demonstrating the effect of rhinovirus challenge on the concentration of CuZnSOD and MnSOD protein in respiratory epithelial cells. Shown in the figure are lanes loaded with either 50 or 80 µg of protein from either virus challenged (V) or control (C) cells.

Effect of Rhinovirus Challenge on SOD Protein and mRNA Concentration

Western and northern blot analysis were done to determine if the virus-induced increase in the specific activity of CuZnSOD was the result of true induction or activation of the pre-existing protein. Immunoblot of cells collected 6 h after rhinovirus challenge revealed that the CuZnSOD concentration was increased in virus challenged cells compared to uninfected controls while no change was observed in MnSOD levels (Fig. 2). Consistent with the enzyme activity and the protein content, CuZnSOD mRNA levels were increased in RV-challenged cells (Fig. 3). There was approximately a two-fold increase in mRNA content 6 h after virus challenge and about a four-fold increase 24 h after challenge compared to unchallenged cells.

DISCUSSION

These results reveal that rhinovirus challenge of respiratory epithelial cells does not reduce the expression or function of cellular antioxidant enzymes during the early phase of virus-induced IL-8 elaboration. The activity of CuZnSOD in challenged cells was increased rather than decreased. These results suggest that rhinovirus-induced oxidant stress is due to prooxidant effects of the virus-cell interaction and is consistent with previous observations that an NADPH-oxidase-like enzyme

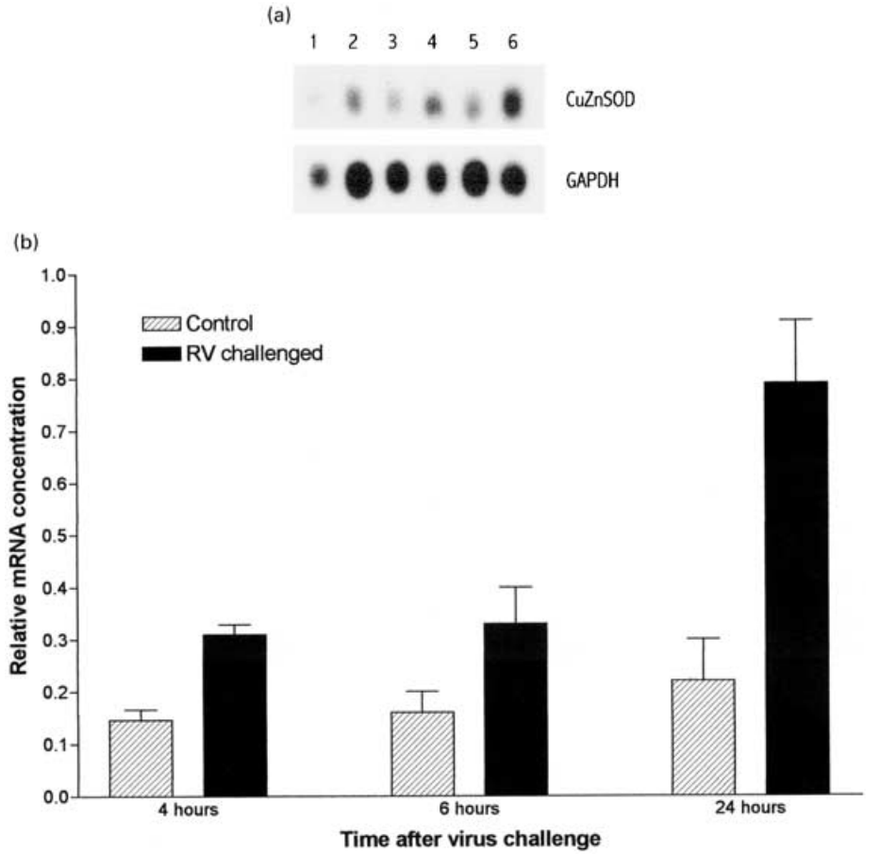

FIGURE 3 Northern blot demonstrating the effect of rhinovirus challenge on the concentration of CuZnSOD mRNA over time in respiratory epithelial cells (a, top panel). The concentration of CuZnSOD was standardized against the concentration of glyceraldehyde-3 phosphate dehydrogenase (a, bottom panel). Concentrations were compared at 4 h (lanes 1 and 2), 6 h (lanes 3 and 4) and 24 h (lanes 5 and 6) after virus challenge (challenged cells are shown in lanes 2, 4 and 6 and control cells are shown in lanes 1, 3 and 5). The change in relative concentration of CuZnSOD mRNA as determined by densitometry is also shown (b).

may play a role in this process. This study addressed the role of alteration of antioxidant enzymes in the production of virus-induced oxidative stress early in the virus replication cycle. It is possible that effects on either the expression or function of these enzymes could be demonstrated as rhinovirus replication proceeds, however, it is unlikely that later effects, if present, would help explain the mechanism of the early phase (2–6 h after challenge) elaboration of IL-8.

The results of this study appear to contrast with studies involving other viral pathogens in other cell lines. HIV infection is associated with significant impairment of antioxidant defense and treatment of patients with *N*-acetyl cysteine has been reported to have a beneficial effect in these patients.[18] Similarly, mice infected with influenza A virus have a significant decrease in total lung glutathione content.[19] It is not clear, however, whether the effects of antioxidant defense described in these studies represent a primary effect of the virus-cell interaction or whether these changes are secondary to the host response to the infection.

The effect of virus challenge on the superoxide dismutases of the host cell appears to differ with different viral pathogens. Challenge of cells with influenza A or Sindbis virus results in selective induction of mitochondrial MnSOD with no change in CuZnSOD.[20,21] In contrast, infection of mouse hearts with encephalomyocarditis virus results in induction of both MnSOD and CuZnSOD.[22] One potential explanation for these different results may relate to the site of superoxide production in response to different stimuli. Influenza infection is associated with production of tumor necrosis factor α.[23] This cytokine stimulates oxidative stress by inhibiting mitochondrial respiration at site II, the site of superoxide production.[24] Thus, induction of mitochondrial MnSOD may be a response to the oxidative stress in this organelle. In contrast, rhinovirus infection is not associated with elaboration of tumor necrosis factor (authors' unpublished observations) and the prooxidant effect of rhinovirus appears to involve a membrane associated oxidase.[5] Thus, the induction of cytosolic CuZnSOD by rhinovirus infection may be an

adaptive defense against the superoxides produced in the cytosol by challenge with this virus.

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