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Cysteine oxidation impairs systemic glucocorticoid responsiveness in children with difficult-to-treat asthma

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Abstract

Background—The mechanisms underlying glucocorticoid responsiveness are largely unknown. Although redox regulation of the glucocorticoid receptor **(GR)** has been reported, it has not been studied in asthma.

Objective—We characterized systemic cysteine oxidation and its association with inflammatory and clinical features in healthy children and children with difficult-to-treat asthma. We hypothesized that cysteine oxidation would be associated with increased markers of oxidative stress and inflammation, increased features of asthma severity, decreased clinically defined glucocorticoid responsiveness, and impaired GR function.

Methods—Peripheral blood mononuclear cells were collected from healthy children (n = 16) and children with asthma (n = 118) age 6-17 years. Difficult-to-treat asthmatic children underwent glucocorticoid responsiveness testing with intramuscular triamcinolone. Cysteine, cystine, and inflammatory chemokines and reactive oxygen species (ROS) generation were quantified and expression and activity of the GR was assessed.

Results—Cysteine oxidation was present in children with difficult-to-treat asthma and was accompanied by increased ROS generation and increased *CCL3* and *CXCL1* mRNA expression. Children with the greatest extent of cysteine oxidation had more features of asthma severity including poorer symptom control, greater medication usage and less glucocorticoid responsiveness despite inhaled glucocorticoid therapy. Cysteine oxidation also modified the GR protein by decreasing available sulfhydryl groups and decreasing nuclear GR expression and activity.

Conclusions—A highly oxidized cysteine redox state promotes a post-translational modification of the GR that may inhibit its function. Given that cysteine oxidation is prevalent in children with

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difficult-to-treat asthma, the cysteine redox state may represent a potential therapeutic target for the restoration of glucocorticoid responsiveness in this population.

Keywords

Biomarker; Childhood asthma; Treatment response; Cysteine; Inflammation; Oxidative stress; Refractory asthma; Severe asthma

INTRODUCTION

Glucocorticoids are the cornerstone of treatment for persistent asthma,[1] but the response to these medications is highly variable, particularly when administered at moderate-to-high-dosages.[2] Whereas several large studies have shown that doubling the dose of inhaled glucocorticoids is of limited efficacy in asthmatic patients already receiving low-dose inhaled glucocorticoid therapy,[3, 4] other studies suggest that quadrupling the dose may improve measures of asthma impairment and prevent exacerbations.[5] However, chronic inflammation may still persist in a subset of patients with more severe disease[6] and the mechanisms underlying decreased glucocorticoid responsiveness in this population remain largely unknown.

Although the biology of the glucocorticoid receptor **(GR)** is complex, post-translational modifications of the GR and associated downstream effects on glucocorticoid signaling have been described.[7] While much of the literature has focused on serine phosphorylation, the function of the GR is also regulated by redox-dependent mechanisms[8, 9] which may be of relevance in asthma. Indeed, we have previously reported marked redox abnormalities in the airways and systemic circulation of children with asthma that worsen with increased asthma severity.[10-15] Notably, in children with symptomatic asthma despite glucocorticoid therapy, the presence of reactive oxygen species promotes oxidation of the amino acid thiol, cysteine, to its disulfide form (i.e., cystine) and further depletes the pool of cysteine available for signal transduction and other cellular functions.[12, 13] Given that cysteine oxidation also stimulates cytokine secretion[16] and promotes pathways associated with cellular stress and death,[17] we sought to: 1) characterize systemic cysteine oxidation and its intracellular, inflammatory and clinical features (including glucocorticoid responsiveness) in children with difficult-to-treat asthma, and 2) explore the role of the cysteine oxidation in GR function. We hypothesized that in children with difficult-to-treat asthma, greater cysteine oxidation would be associated with increased intracellular and extracellular oxidative stress and inflammation, increased features of asthma severity, and decreased glucocorticoid responsiveness. We further hypothesized that cysteine oxidation would impair nuclear GR expression and consensus binding.

METHODS

Participants

Children 6 to 17 years of age with physician-diagnosed asthma who were receiving current treatment for asthma were enrolled from a difficult-to-treat asthma clinic at Emory University in Atlanta, Georgia. Asthmatic children had a history of ≥ 12% reversibility in

the forced expiratory volume in one second **(FEV_1)** after short-acting bronchodilator administration or airway hyperresponsiveness to methacholine evidenced by a methacholine PC_{20} ≤16 mg/mL. Healthy children without asthma were also enrolled for comparison. Exclusion criteria for all children included premature birth before 35 weeks gestation, current smoking, immunodeficiency, pulmonary aspiration disorders or vocal cord dysfunction. The Emory University Institutional Review Board granted approval for this study. Written informed consent was obtained from the parents or legal guardians. Children 12 to 17 years provided written informed assent, whereas children 6 to 11 years provided verbal assent.

Participant characterization procedures

Children were evaluated during two visits separated by two weeks. The baseline characterization visit was rescheduled if upper respiratory viral symptoms, acute worsening of asthma symptoms, antibiotic use, or systemic glucocorticoid use was reported within the preceding two weeks. Parents completed medical history questionnaires and children completed the Asthma Control Questionnaire **(ACQ)** and the Pediatric Asthma Quality of Life Questionnaire **(PAQLQ)**.[18, 19] Spirometry (KoKo PDS, Ferraris, Louisville, CO) was performed at baseline and after receipt of up to eight inhalations of albuterol sulfate (90 μg/actuation). The best of three forced vital capacity **(FVC)** maneuvers was interpreted.[20] Exhaled nitric oxide concentrations were determined using online methods (NIOX MINO®, Aerocrine, Morrisville, NC).[21] Whole blood (up to 25 mL) from venipuncture was collected into serum separation tubes and heparinized tubes containing a density gradient for peripheral blood mononuclear cell **(PBMC)** isolation (Vacutainer® CPT™, Becton, Dickinson and Company, Franklin Lakes, NJ). Total serum immunoglobulin E **(IgE)** was quantified with an assay kit according to the manufacturer's instructions (Calbiotech, Spring Valley, CA).

Glucocorticoid responsiveness testing

At the completion of baseline visit, a subset (n = 57) of participants with symptomatic asthma despite moderate-to-high dose inhaled glucocorticoid therapy (i.e., >200 μg fluticasone equivalent for children 6-11 years and >500 μg fluticasone equivalent for children 12 years and older) received intramuscular triamcinolone (1 mg/kg, 60 mg maximum dose). Symptomatic asthma was defined according to available treatment guidelines[1] as self-reported asthma symptoms more than twice weekly or nocturnal awakenings from asthma at least 2 nights per month. Responsiveness to triamcinolone was assessed after two weeks and was defined as an ACQ score < 0.75, which corresponds to "well-controlled asthma" with a positive predictive value and negative predictive value of 0.73 and 0.85, respectively.[22]

Cysteine and cystine determination

To prevent auto-oxidation prior to analysis, aliquots were preserved for in a 5% perchloric acid solution containing iodoacetic acid (6.7 μmol/L) and boric acid (0.1 mol/L) with 5 μmol/L γ-glutamyl-glutamate internal standard.[23] Cysteine and cystine were quantified relative to γ-glutamyl-glutamate by reverse-phase high-performance liquid chromatography

as described previously.[12] Samples were derivatized with dansyl chloride and separated on a 10 μm Ultrasil amino-acid column (Waters Alliance 2690, Waters Corporation, Milford, MA). Fluorescence was detected at 365 and recorded by two detectors (Waters 474, Waters Corporation, and Gilson 121, Gilson Inc., Middletown, WI). The redox potential **(E_h)** of the cysteine/cystine thiol pair was calculated with the Nernst equation, $E_h = E_o + RT/nF \ln$ [disulfide]/([thiol1][thiol2]), where E_o is the standard potential for the redox couple (−250 mV), R is the gas constant, T is the absolute temperature, n is the number of electrons transferred, and F is the Faraday constant.

Cellular viability and reactive oxygen species generation

Cellular viability was determined using an automatic cell counter (Countess®, Invitrogen, Grand Island, NY) after staining with 0.4% trypan blue. Intracellular reactive oxygen species **(ROS)** generation was assessed after incubation with 5 μM 2′,7′-Dichlorodihydrofluorescin diacetate for 45 minutes. Relative fluorescent units **(RFU)** were quantified in 5-10 separate visual fields with FluoView software (Olympus; Center Valley, PA) after correction for background autofluorescence.

Real-time polymerase chain reaction

Cells were added to 5 volumes of RNA*later*® (Life Technologies, Grand Island, NY) and RNA was extracted using a commercial kit (RNeasy® Mini kit RNeasy Mini, Qiagen, Valencia, CA). 10ng of total RNA per sample was reverse transcribed using MultiScribe® Reverse Transcriptase (62.5U/50ul reaction), RNase Inhibitor, oligoT primers and MgCl2 at a concentration of 5.5mM (Life Technologies). cDNA aliquots were preamplified for genes of interest using TaqMan PreAmp Master Mix Kit (Life Technologies). The pre-amplified cDNA was used to quantitate relative levels of *CCL3* (Hs00234142_m1) and *CXCL1* (Hs00236937_m1) in a 96-well assay system (StepOnePlus™ real-time PCR assay) with TaqMan® primer pairs and probes (Life Technologies). Data were normalized to *B2M* (4333766F), *GAPDH* (4333764F), *ACTB* (4333762F), *PGK1* (4333765F), and *PPIA* (43337663F) housekeeping genes. Net cycle threshold **(CT)** values were used to calculate ΔCT values for each subject, using the average of the five housekeeping genes as a reference. mRNA gene expression was analyzed relative to the control group and was expressed as fold change values.

Cell culture

Cell culture experiments were performed with THP-1 monocytes (ATCC®, Manassas, VA), MM.1S B lymphoblasts (ATCC®) and primary PBMCs from healthy donors (AllCells, Alameda, CA). Reduced and oxidized conditions were created by adding 300 μL of 10 mM cysteine and 150 μL of 10 mM cystine (Sigma-Aldrich, St. Louis, MO), respectively, to 15 mL DMEM/F12 media without methionione, cysteine, cystine and glutamine (HyClone, Life Technologies). Media was supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (Cellgro, Corning Life Sciences, Corning, NY), and 50 mg/mL gentamicin sulfate (Cellgro). Cells were re-suspended to a concentration of 1 million cells/mL and cultured for 4 hours at 37°C with 5% CO_2. In selected experiments, cells were also exposed to 100 nM dexamethasone for 1 hour (Sigma-Aldrich), added at hour 3 of incubation.

Western blotting

Cells were suspended in 50 mM Bis-Tris-HCL lysis buffer, pH=6.5, containing 0.5% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 150mM NaCl, 1 mM EDTA and 0.1 mM PMSF. Protein sulfhydryl **(−SH)** group protein residues were labeled with biotinylated iodoacetamide using the methods of Go et al.[24] at a final concentration of 20 μM for 15 minutes, after which iodoacetamide was added to a final concentration of 5 mM. The GR was immunoprecipated using an anti-GR receptor antibody (Santa Cruz Biotechnology, Dallas, TX) and the Protein G Immunoprecipitation Kit (Sigma-Aldrich). Eluted sample was divided and run on two 10% SDS-PAGE gels. Proteins were transferred overnight to nitrocellulose membranes and biotinylated iodoacetamine binding was visualized on an Odyssey® Classic Infrared Imaging System (LI-COR, Lincoln, NE) by incubating with streptavidin-conjugated IRDye® 680RD (LI-COR) for one hour. Even loading of samples was determined by incubating the second membrane overnight at 4°C with human GR antibody raised in rabbit (Santa Cruz Biotechnology). Visualization was performed after secondary incubation with anti-rabbit IgG conjugated to IRDye® 680RD (LI-COR).

Nuclear isolation, total protein measurement and nuclear GR expression and activation

Nuclei were isolated using a commercial nuclear extract kit (Active Motif, Carlsbad, CA). Protein concentrations were measured at 750nm using bovine serum albumin as the standard (DC™ Protein Assay Kit, Bio-Rad, Hercules, CA). Equal nuclear protein volumes were added to a TransAM™ assay plate (Active Motif) coated with immobilized oligonucleotide containing the GR consensus binding site (5′-GGTACAnnnTGTTCT-3′). GR activity was assessed per the manufacturer's instructions with an optical density of 450 nm. SDS-PAGE was performed using 30 μg of nuclear lysate loaded onto a precast gradient (4-20%) gel (Bio-Rad). Proteins were transferred onto a nitrocellulose membrane (Bio-Rad) and incubated with human GR antibody raised in rabbit (Santa Cruz) and IRDye 800CW Donkey anti-Rabbit IgG and IRDye 680RD Donkey anti-Goat IgG (Li-Cor Biosciences, Lincoln, NE). Membranes were visualized on an Odyssey Classic Infrared Imaging System and densitometry performed using Image Studio Lite version 4.0 (Li-Cor Biosciences).

Statistical analyses

Statistical analyses were performed with IBM® SPSS® Statistics software (Version 22, SPSS Inc, Chicago, Illinois). Chi-square tests were used for dichotomous variables. T-tests and analysis of variance with Fishers Least Significance Difference post-hoc tests were used for variables that were normally distributed. For skewed variables, statistical significance testing was performed with non-parametric Mann-Whitney U tests or Kruskal-Wallis tests, as appropriate. Bivariate Pearson correlations were used to examine associations between linear variables. Logistic regression analyses were performed to determine the associations between tertiles of plasma cysteine/cystine redox potentials and clinical outcomes. Univariate models were first used to narrow the list of covariates (statistically significant at $p < 0.05$) to be incorporated into the final multivariate model. Significance was defined as $\alpha < 0.05$ using two-tailed tests.

RESULTS

One hundred thirty four children were enrolled for this study but 20 were excluded due to an inadequate sample, leaving 114 children (healthy control, n = 15; asthma, n = 99) in the final analysis (Online Repository, Figure E1). Features of the excluded children were not significantly different (12 ± 4 years; 15% White; 45% with BMI percentile <85%; 93 ± 13% predicted FEV_1, $p > 0.30$ for each). Features of the included participants are shown in Table 1. Children with asthma were more likely to be non-white males and were further characterized by greater allergic sensitization, more airflow limitation, and increased airway inflammation as reflected by exhaled nitric oxide values, consistent with their asthma diagnosis. Children with asthma were also more obese with higher body mass index percentiles.

Characterization of systemic cysteine oxidation

Compared to healthy non-asthmatic children, children with asthma had lower baseline plasma concentrations of cysteine and higher plasma concentrations of the disulfide, cystine, resulting in a more oxidized cysteine/cystine redox potential (Figure 1). Extracellular cysteine oxidation in the asthmatic participants was further accompanied by increased intracellular glutathione disulfide formation, increased reactive oxygen species generation and increased mRNA expression of the pro-inflammatory genes, *CCL3* and *CXCL1* (Figure 2). Although all children with asthma were difficult-to-treat, children with severe asthma defined by the need for high-dose inhaled glucocorticoids plus an additional controller medication to maintain asthma control[25] had the lowest plasma cysteine concentrations and the most oxidized cysteine/cystine redox potential (Online repository, Figure E2).

Associations between systemic cysteine oxidation and asthma clinical features

Given the heterogeneity in extracellular cysteine/cystine redox potentials observed within the group of asthmatic participants, associations between tertiles of plasma cysteine/cystine redox potentials and asthma clinical features were explored. Asthmatic children with plasma cysteine/cystine redox potentials in the highest (i.e., most oxidized) tertile had over three-fold increased odds of high-dose inhaled glucocorticoid therapy and daily short-acting beta-agonist therapy compared to children in the lowest (i.e., more reduced) tertile. Moreover, children in the highest tertile also had evidence of poorer current and historical asthma control, reflected by ACQ scores greater than 1.5 at the characterization visit[22] and more frequent emergency department visits and intubations (Online Repository, Table E1).

Associations between systemic cysteine oxidation and glucocorticoid responsiveness

To further understand associations between systemic cysteine oxidation and features of asthma severity in children, we determined whether the baseline plasma cysteine/cystine redox potential was also associated with clinical responsiveness to systemic glucocorticoids. For this analysis, a subset of asthmatic participants with poor asthma control despite treatment with moderate-to-high doses of inhaled glucocorticoids (n = 57) received intramuscular triamcinolone and returned to the clinic for re-evaluation in two weeks. Features of the children who received triamcinolone are presented in **Table E2 (Online Repository)**.

At the two-week visit, the prevalence of study-defined triamcinolone responsiveness and non-responsiveness was 59% (n = 33) and 41% (n = 23), respectively. In addition to having poorer asthma control, asthmatic children who did not respond to triamcinolone also had more impaired asthma-related quality of life, increased exhaled nitric oxide concentrations, and greater pre-bronchodilator airflow limitation after the triamcinolone injection (Table 2). Furthermore, the plasma cysteine/cystine redox potential was higher (i.e., more oxidized) in triamcinolone non-responders at both the baseline visit and the two-week characterization visit (Figure 3) due to lower cysteine concentrations in the non-responders at both time points (responders vs. non-responders, baseline cysteine: 1.32 ± 1.50 vs. 0.48 ± 0.52 μM, p = 0.013; 2-week cysteine: 1.33 ± 1.31 vs. 0.58 ± 0.79 μM, p =0.40). Plasma concentrations of cystine, mixed disulfides, glutathione and glutathione disulfide did not differ between groups (data not shown). Stratification of participants by baseline tertiles of plasma cysteine/cystine redox potentials also demonstrated increased odds of triamcinolone non-responsiveness at the follow-up visit in the most oxidized group (OR for highest versus lowest tertile: 5.83; 95% CI: 1.06 – 32.02, p = 0.042). Overall, the plasma cysteine/cysteine redox potential for each participant, including healthy controls and children who did not receive triamcinolone, was relatively stable (Online Repository, Figure E4). mRNA gene expression of *CCL3* and *CXCL1*was also increased in triamcinolone non-responders compared to triamcinolone responders (Online Repository, Figure E5), consistent with the clinical observations of increased inflammation in these children (Table 2).

Proof-of-concept mechanistic studies

Because the volume of samples available from children was limited, proof-of-concept experimentation was also performed using THP-1 monocytes and primary PBMCs from healthy donors. Exposure of these cells to extracellular oxidizing versus reducing cysteine/cystine environments resulted in physiologically relevant redox potentials and differences in intracellular glutathione disulfide and ROS generation (Online Repository, Figure E6) compared what we previously observed in our pediatric participants. Oxidizing extracellular conditions were also associated with decreased availability of GR protein −SH groups for binding (Online Repository, Figure E7, A-B). Similar decreases in GR protein −SH group availability were observed samples from difficult-to-treat asthmatic children (Online Repository, Figure E7, C-D). Furthermore, GR activity, reflected by decreased GR consensus binding, was blunted in response to extracellular oxidizing conditions (Figure 4, A-B). Additional experiments with MM.1S cells, which naturally over-express the GR, further revealed decreased expression of the GR in the nucleus with extracellular cysteine/cystine oxidation.

DISCUSSION

To our knowledge, this is the first study to characterize cysteine oxidation and its association with glucocorticoid responsiveness in patients with difficult-to-treat asthma. Although heterogeneity in our results was noted, a significant degree of cysteine oxidation was noted in a subset of children with difficult-to-treat asthma and was accompanied by increased ROS generation and increased mRNA expression of *CCL3* and *CXCL1*. This heterogeneity was not clearly attributable to obesity or inhaled glucocorticoid exposure, although children with

the greatest extent of cysteine oxidation did have more features of asthma severity including poorer symptom control, greater medication usage including use of high-dose inhaled glucocorticoids, and decreased clinically defined glucocorticoid responsiveness. While the precise mechanisms underlying these observations are unclear, our proof-of-concept experiments suggest that cysteine oxidation promotes a post-translational modification of the GR that impairs its nuclear expression and binding to the glucocorticoid consensus.

Clinically-defined impairments in glucocorticoid responsiveness have been previously reported in patients with asthma, albeit there are no gold standard definitions for glucocorticoid “response” and methodologies vary widely across studies.[26] A recent unbiased cluster analysis of children enrolled in the Childhood Asthma Management Research Program identified two clusters of childhood asthma with positive versus minimal responses to inhaled budesonide that differed significantly with regard to the rate of exacerbations, the number of controller medications for asthma, and longitudinal differences in pulmonary function.[27] A separate study of children with difficult-to-treat asthma similarly identified several groups of patients with varying degrees of responsiveness to intramuscular triamcinolone.[28] In that study, only 11% of enrolled children had “complete” responsiveness to triamcinolone as determined by cut-points of symptoms, FEV1, bronchodilator reversibility and exhaled nitric oxide.[28] Rather, the majority of children were “partial” or “incomplete” responders,[28] highlighting the heterogeneity of the disorder as it is currently defined. It is also not clear how clinical end points such as lung function or symptoms associate with airway inflammation or other biological mechanisms that may underlie asthma phenotypes.

While the biological mechanisms that regulate the response to glucocorticoids are not entirely clear, other studies have described reduced binding of glucocorticoid to its receptor and reduced GR consensus binding in these patients[29, 30] that might be related to persistent activation of p38 mitogen-activated protein kinase.[31, 32] Other studies have reported increased expression of the GR beta isoform in airway cells,[33] immune cells[34] and airway tissue[35] of patients with severe asthma that may act as a negative inhibitor and compete for binding of GR consensus. Abnormal histone acetylation has also been reported in patients with severe asthma[36] and COPD[37] and is characterized by a reduction in histone deacetylase 2 expression and activity, which may be induced by oxidative and nitrosative stress.[38, 39]

Like any protein, the GR contains a number of cysteine residues that maintain its structure and function. The hormone binding domain of the GR (residues 529-777 in humans) contains 13 methionines but only 5 cysteines **(Cys)** at residues 622, 638, 643, 665, and 736 that are sensitive to changes in redox status.[40] Under oxidizing conditions, Cys-622, 638, and 643 form disulfide bonds[41-43] that may cause the GR to assume a folded conformation.[44] Additionally, disulfide bonds with Cys-481 in the DNA binding domain of the GR have been shown to inhibit nuclear translocation, presumably through altered GR protein conformation.[9] Thus, redox regulation of the GR is most likely due to functional alteration of the cysteine residues of the GR in the presence of reactive oxygen species, and not to a decrease in expression of the GR protein.[45-47]

This study does have a number of limitations. Although adherence to inhaled glucocorticoids cannot be guaranteed in our participants with difficult-to-treat asthma, intramuscular triamcinolone removes some issues with inhaled glucocorticoid delivery given its depot effect.[26] While previous studies utilizing triamcinolone in children have demonstrated overall reductions in inflammatory biomarkers and asthma symptoms after the injection, variability in the clinical response was noted.[28, 48] Although a higher dose of triamcinolone may have conferred additional benefit,[49] these benefits are likely to be outweighed by the systemic side effects of the medication in school age children who are still undergoing linear growth. Furthermore, while the use of PBMCs can be criticized, our previous work[12] and that of others[34] has shown similarities between PBMCs and airway cells in patients with severe asthma whose inflammation is not limited to the airways. The use of PBMCs also allowed for a control group that otherwise could not be obtained due to ethical reasons.

In conclusion, we have shown that oxidation of the amino acid, cysteine, is associated with decreased responsiveness to systemic glucocorticoids in children with difficult-to-treat asthma. While additional mechanistic studies are warranted, our findings also suggest that redox-related mechanisms can impair GR function in asthmatic patients. While glucocorticoid responsiveness is likely associated with multiple mechanisms (including mechanisms independent of oxidative stress), the cysteine redox state may represent one of several potential therapeutic targets for the restoration of glucocorticoid responsiveness in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ACQ	Asthma Control Questionnaire
CT	Cycle threshold
Cys	Cysteine residue
E_h	Redox potential
FEF_{25-75}	Mid-expiratory flow rate at 25-75% of vital capacity
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity

GR	Glucocorticoid receptor
IgE	Immunoglobulin E
PAQLQ	Pediatric Asthma Quality of Life Questionnaire
PBMC	Peripheral blood mononuclear cell
PC_{20}	Provocative concentration of methacholine resulting in a 20% decline in FEV_1
RFU	Relative fluorescent units
ROS	Reactive oxygen species
−SH	Sulfhydryl group

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CLINICAL IMPLICATIONS

Systemic oxidative stress, reflected by oxidation of the amino acid, cysteine, is associated with poor asthma control and decreased responsiveness to systemic glucocorticoids in children with difficult-to-treat asthma.

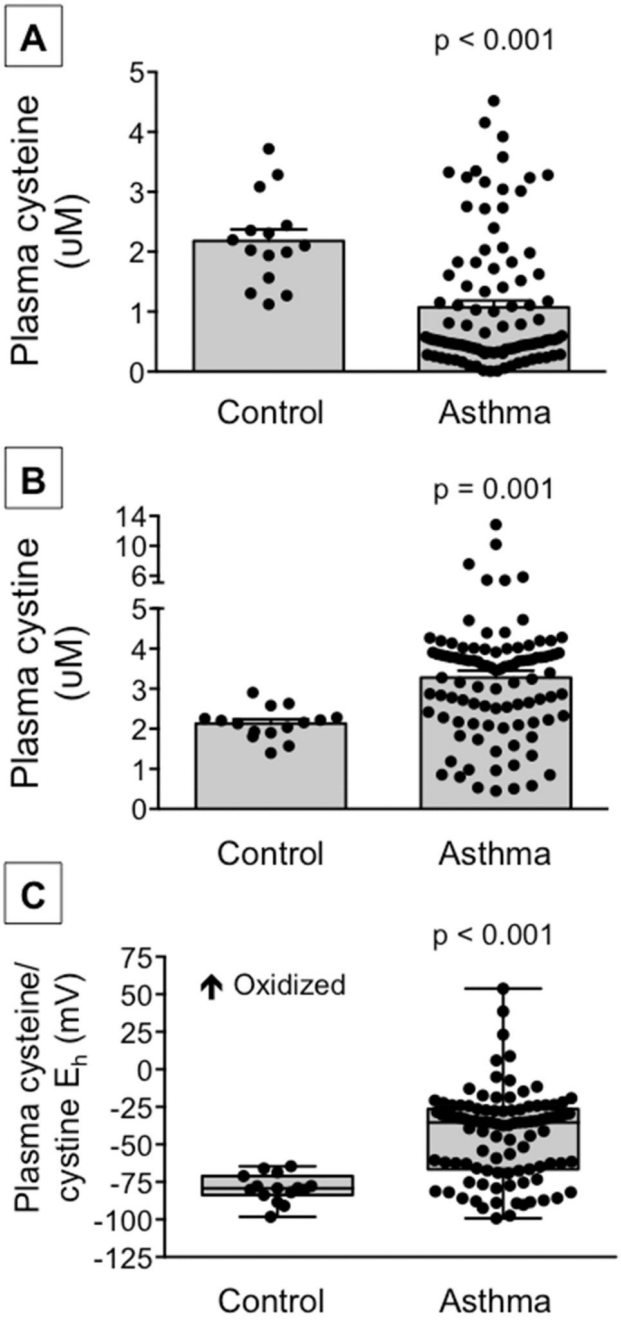

Figure 1.

Plasma (A) cysteine and (B) cystine concentrations and (C) the cysteine/cystine redox potential (E_h) in healthy control children and children with asthma. Cysteine and cystine data are presented as the mean ± SEM and the cysteine/cystine E_h boxplots are shown with minimum and maximum values. Dots represent individual participants. Control: n = 15, asthma: n = 99 for each panel.

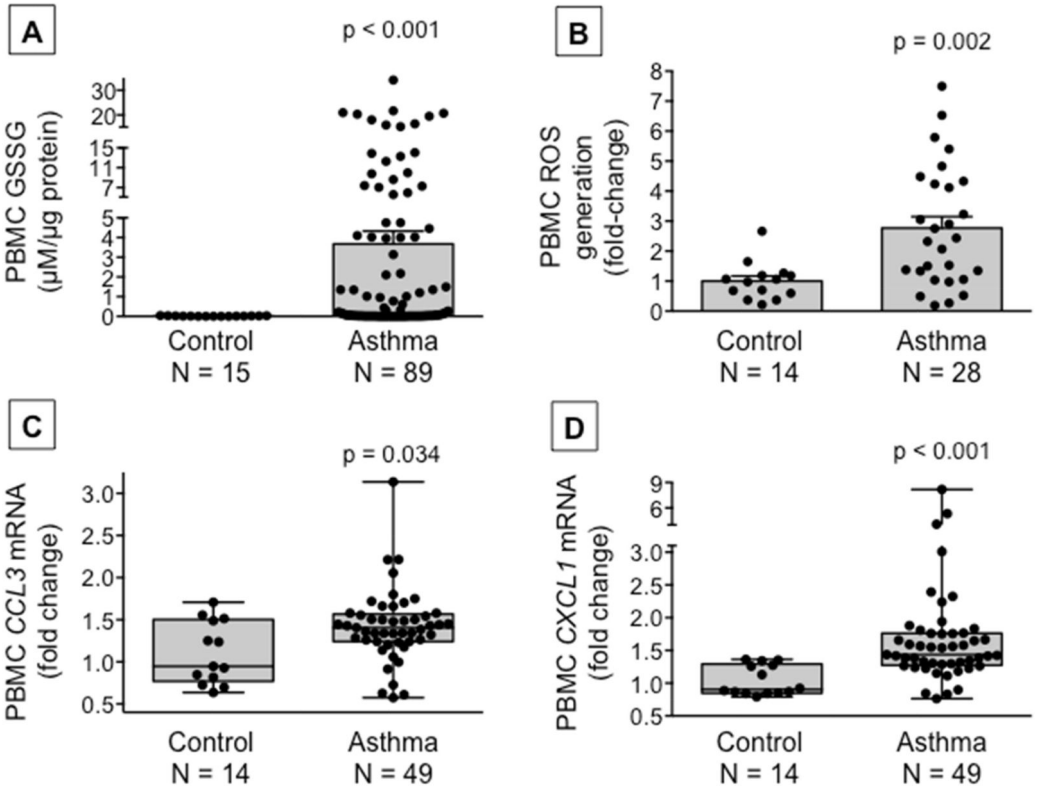

Figure 2.

Peripheral blood mononuclear cell (A) glutathione disulfide (GSSG) concentrations, (B) reactive oxygen species (ROS) generation, and (C) *CCL3* and (D) *CXCL1* mRNA gene expression in healthy control children and children with asthma. GSSG and ROS data are presented as the mean ± SEM and *CCL3* and *CXCL1* mRNA data are presented as boxplots with minimum and maximum values. Dots represent individual values.

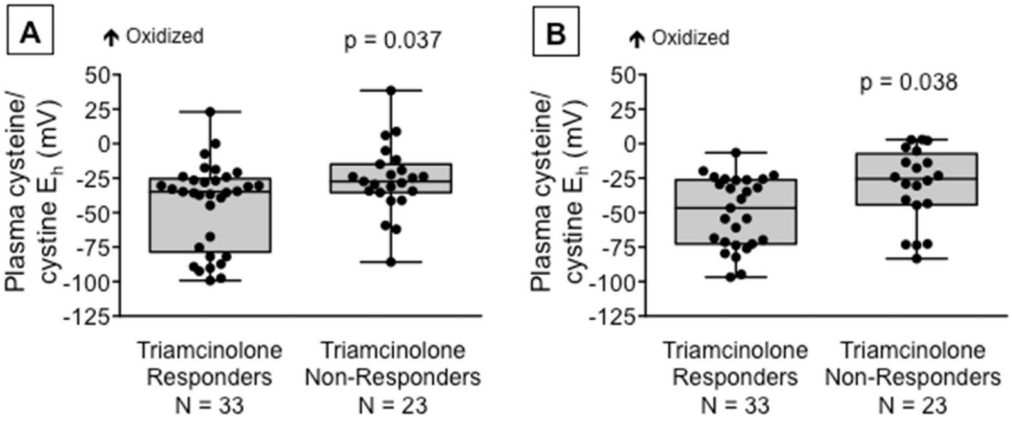

Figure 3.

The plasma cysteine/cystine redox potential (E_h) in triamcinolone responders and triamcinolone non-responders at (A) the baseline visit prior to triamcinolone receipt and (B) two weeks after triamcinolone administration. Triamcinolone "response" was defined as an Asthma Questionnaire Control Score <0.75 at the two-week visit.[22] Boxplots are shown with minimum and maximum values. Dots represent individual values.

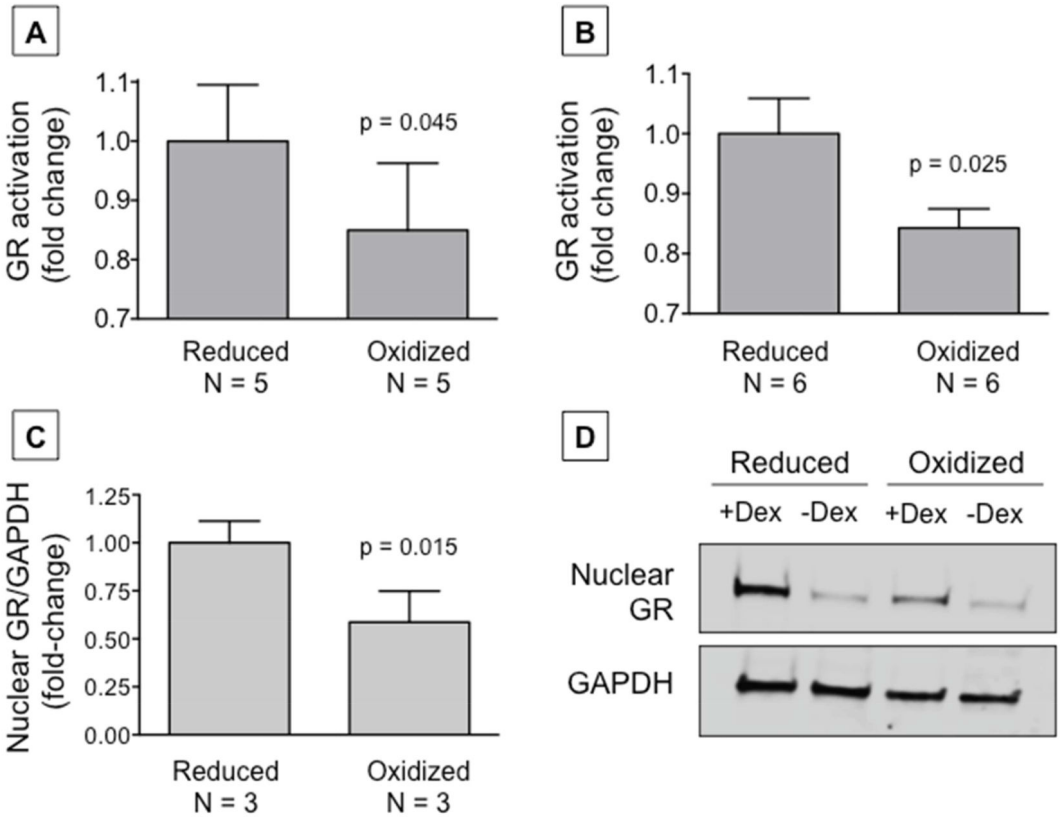

Figure 4.

Glucocorticoid receptor (GR) activation in (A) THP-1 monocyes and (B) human primary PBMCs and (C) nuclear GR expression in MM.1S cells after exposure to extracellular oxidizing versus reduced conditions. GR densitometry is normalized to glyceraldehyde-3-phosphate (GAPDH). Representative images of nuclear GR expression with (+) and without (−) dexamethasone (Dex) treatment are shown in panel (D).

Table 1

Features of the included participants. Data represent the median (IQR) or the number of participants (%).

Feature	Healthy controls N = 15	Asthmatics N = 99	p-value
Age (years)	10 (9, 13)	12 (10, 15)	0.175
Sex			
Male	3 (20)	66 (67)	0.001
Female	12 (80)	33 (33)	
Race			
White	3 (20)	16 (16)	0.001
Black	9 (60)	69 (70)	
Asian	3 (20)	1 (1)	
More than one race	0	13 (11)	
Body mass index			
< 85th percentile (normal weight)	9 (82)	43 (43)	0.036
85th to 95th percentile (overweight)	2 (18)	26 (26)	
≥ 95th percentile (obese)	0	30 (30)	
Self-reported allergic rhinitis	10 (67)	91 (92)	0.004
Serum IgE (kU/L)	61 (30, 94)	218 (40, 503)	0.011
Exhaled nitric oxide (ppb)	12 (9, 14)	29 (17, 51)	< 0.001
Baseline lung function			
FVC (% predicted)	102 (88, 110)	105 (98, 115)	0.652
FEV_1 (% predicted)	98 (89, 108)	93 (81, 105)	0.046
FEV_1/FVC	0.87 (0.83, 0.93)	0.78 (0.70, 0.83)	< 0.001
FEV_1/FVC (% predicted)	101 (95, 109)	90 (81, 95)	< 0.001
FEF_{25-75} (% predicted)	92 (74, 112)	71 (56, 86)	< 0.001
Post-bronchodilator lung function			
FVC (% predicted)	104 (90, 121)	110 (102, 120)	0.294
FEV_1 (% predicted)	108 (93, 118)	105 (93, 115)	0.705
FEV_1/FVC	0.90 (0.83, 0.96)	0.84 (0.78, 0.88)	0.004
FEV_1/FVC (% predicted)	104 (94, 110)	97(91, 101)	0.010
FEF_{25-75} (% predicted)	108 (91, 137)	94 (81, 110)	0.161

Table 2

Features of triamcinolone responders versus non-responders at the two-week follow-up visit.[1] Triamcinolone response was defined as an Asthma Control Questionnaire score < 0.75.[22] Data represent the median (IQR).

Feature	Triamcinolone responder N = 33	Triamcinolone non-responder N = 23	p-value
Pediatric Asthma Quality of Life Questionnaire total score[2]	6.56 (6.25, 6.83)	5.41 (4.45, 6.01)	< 0.001
Exhaled nitric oxide (ppb)[3]	14 (6, 25)	26 (12, 45)	0.040
Baseline lung function			
FVC (% predicted)	105 (99, 115)	92 (84, 117)	0.038
FEV_1 (% predicted)	96 (87, 102)	80 (69, 101)	0.007
FEV_1/FVC	0.78 (0.72, 0.87)	0.75 (0.67, 0.80)	0.207
FEV_1/FVC (% predicted)	90 (85, 101)	87 (78, 92)	0.093
FEF_{25-75} (% predicted)	75 (61,91)	56 (45, 76)	0.016
Post-bronchodilator lung function			
FVC (% predicted)	109 (103, 120)	101 (93, 117)	0.111
FEV_1 (% predicted)	105 (98, 114)	96 (88, 114)	0.085
FEV_1/FVC	0.85 (0.79, 0.91)	0.85 (0.81, 0.87)	0.692
FEV_1/FVC (% predicted)	98 (92, 106)	98 (95, 100)	0.523
FEF_{25-75} (% predicted)	99 (83, 113)	94 (72, 109)	0.157

[1] One participant did not complete the follow up visit and was excluded from this comparison.

[2] Scores on the Pediatric Asthma Quality of Life Questionnaire range from 0 to 7, with higher scores indicating better quality of life.

[3] Data were logarithmically transformed prior to statistical analysis.