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Enhancement of systemic and sputum granulocyte response to inhaled endotoxin in people with the GSTM1 null genotype

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Abstract

Objective—To determine if the GSTM1 null genotype is a risk factor for increased inflammatory response to inhaled endotoxin.

Methods—35 volunteers who had undergone inhalation challenge with a 20 000 endotoxin unit dose of Clinical Center Reference Endotoxin (CCRE) were genotyped for the GSTM1 null polymorphism. Parameters of airway and systemic inflammation observed before and after challenge were compared in GSTM1 null (n=17) and GSTM1 (n=18) sufficient volunteers.

Results—GSTM1 null volunteers had significantly increased circulating white blood cells (WBCs), polymorphonuclear neutrophils (PMNs), platelets and sputum PMNs (% sputum PMNs and PMNs/mg sputum) after CCRE challenge. GSTM1 sufficient volunteers had significant, but lower increases in circulating WBCs, PMNs and % sputum PMNs, and no increase in platelets or PMNs/mg sputum. Linear regression analysis adjusted for baseline values of the entire cohort revealed that the GSTM1 null genotype significantly increased circulating WBCs, platelets and % sputum PMNs after challenge

Conclusion—These data support the hypothesis that the GSTM1 null genotype is a risk factor for increased acute respiratory and systemic inflammatory response to inhaled CCRE. These data are consistent with other observations that the GSTM1 null genotype is associated with increased respiratory, systemic and cardiovascular effects linked to ambient air particulate matter exposure

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and indicate that the GSTM1 null genotype should be considered a risk factor for adverse health effects associated with exposure to environmental endotoxin.

INTRODUCTION

Exposure to environmental endotoxin is linked to a number of clinical diseases, including byssinosis, occupational lung disease in animal workers, and asthma exacerbation.¹ Endotoxin is a component of bioaerosols associated with exposure to animals in agricultural and domestic settings, particulates produced in tobacco and biomass fuel smoke, and has been found in coarse and fine mode ambient and indoor air particulate matter (PM).¹ Ambient air PM and passive smoke exposure have been associated with increased respiratory tract² and cardiovascular morbidity.³ Additionally, ambient air PM exposures are associated with increased markers of systemic inflammation.⁴ Taken together, these reports suggest that inhaled PM can exert local effects on the airway and impact other organ systems via increased systemic inflammation. As endotoxin is an important constituent of many bioaerosol and PM species,¹ and is the primary ligand for the TLR4 receptor on macrophages and neutrophils,¹ it is very likely that environmental endotoxin contributes to systemic morbidity in PM exposed populations.

Endotoxin promotes oxidative burst activity and production of reactive oxygen species by phagocytic and innate immune cells, which is thought to be an important mechanism for inducing exacerbation of lung disease.⁵ Inhalation of endotoxin also increases markers of systemic oxidant stress. We hypothesise that the effect of inhaled endotoxin on airway and systemic inflammation will be enhanced in persons with genetic deficiencies in antioxidant defences. A number of polymorphisms of antioxidant genes have been associated with increased response to environmental agents. Among these is a gene deletion (null) polymorphism of the glutathione-S-transferase Mu1 (GSTM1) gene. The homozygous GSTM1 null genotype is prevalent in approximately 40–50% of the population⁶ and has been associated with increased risk for cardiovascular disease events associated with exposure to ambient air PM,⁷ increased risk of wheezing in children exposed to tobacco smoke during the perinatal period, and increased risk of acute exacerbation of asthma related to ambient air O₃ exposure⁶.

We have developed a low dose endotoxin challenge protocol which models occupational and environmental exposure to airborne endotoxin using 20 000 endotoxin units (EU) of Clinical Center Reference Endotoxin (CCRE), which approximates the amount of endotoxin that a worker would be exposed to during an 8 h work shift in a concentrated animal farming operation. We have used this level of inhaled CCRE in studies of normal volunteers and subjects with asthma and find that it causes increased sputum neutrophilia without any fever, systemic symptoms or change in lung function. In this report, we identified 35 healthy volunteers who had undergone inhalation challenge with CCRE and stratified them on the basis of GSTM1 genotype. We compared systemic (circulating) white blood cells (WBCs) and polymorphonuclear neutrophil (PMN) counts and sputum PMNs, expressed as % of total cells that are PMNs (% sputum PMNs) and PMNs/mg sputum, 4 h after challenge of GSTM1 null and sufficient volunteers to test the hypothesis that absence of GSTM1 enhances inflammatory response to inhaled CCRE.

METHODS

Thirty-five healthy non-allergic, adults without asthma who had undergone endotoxin inhalation challenge with a 20 000 EU dose of CCRE between 2003 and 2009 in identical protocols approved by the University of North Carolina IRB were included in this study. All volunteers had normal lung function, normal methacholine challenge and negative skin tests

and were genotyped only after challenge and assessment of samples was completed, thus avoiding selection bias. Venipuncture, induced sputum, spirometry, cytokine measurements in sputum and genotyping for GSTM1 were performed as previously described.⁸⁹ Circulating total WBCs, PMNs and platelet counts, sputum PMNs and mediators in recovered sputum samples, spirometry measures and symptom scores were assessed at baseline (pre-challenge) and 4 h after CCRE challenge was complete in GSTM1 null and sufficient cohorts. Baseline sputum and blood samples were obtained 24–48 h prior to CCRE challenge. CCRE inhalation had no effect on symptoms or spirometry.

To determine if CCRE had an overall effect on circulating and sputum inflammatory responses, data were log-transformed to ensure a normal distribution and paired t tests of pre- and post-endotoxin values were performed. To examine the effect of the GSTM1 null genotype on WBCs, PMNs, platelets and sputum PMNs (% sputum PMNs and PMN/mg sputum) following CCRE challenge across the entire cohort (n=35), we used the log-transformed values of these analytes in a linear regression model after adjusting for baseline analyte values, age, gender, ethnicity and body mass index (BMI).

RESULTS

Figure 1 depicts the levels of baseline and post-CCRE challenge circulating WBCs and PMNs (figure 1A,B), % sputum PMNs (figure 1C) and sputum PMNs expressed as PMN/mg sputum (figure 1D) in the GSTM1 sufficient and null groups. In GSTM1 sufficient volunteers, CCRE significantly increased circulating WBCs (p=0.006) and PMNs (p=0.001), as well as the % sputum PMNs (p=0.01), but not PMN/mg sputum (p=0.11). In GSTM1 null volunteers, CCRE increased circulating WBCs (p=0.001) and PMNs (p=0.003), as well as both sputum measures of PMNs, % sputum PMNs (p=0.002) and PMNs/mg sputum (p=0.005).

We also found a significant CCRE-induced increase in blood platelets in GSTM1 null volunteers ($27.3 \pm 1.8 \times 10^6/\text{ml}$ baseline vs $28.4 \pm 1.8 \times 10^6/\text{ml}$ post-CCRE, p=0.01) but not in GSTM1 sufficient volunteers ($25.3 \pm 1.5 \times 10^6/\text{ml}$ baseline vs $25.8 \pm 1.6 \times 10^6/\text{ml}$ post-CCRE, p=0.41). CCRE induced significant changes in both GSTM1 cohorts in sputum TNF (43 ± 22 pg/ml baseline vs 63 ± 33 pg/ml post-CCRE in GSTM1 sufficient volunteers, p=0.05 and 26 ± 7 pg/ml baseline vs 118 ± 63 pg/ml post-CCRE in GSTM1 null volunteers, p=0.002) and IL-1 levels (369 ± 297 pg/ml baseline vs 517 ± 377 pg/ml post-CCRE in GSTM1 sufficient volunteers, p=0.01 and 234 ± 66 pg/ml baseline vs 598 ± 255 pg/ml post-CCRE in GSTM1 null volunteers, p=0.007). Linear regression analysis adjusted for baseline values revealed that the GSTM1 null genotype significantly increased post-challenge circulating WBCs ($\beta=0.120$, SE=0.059, p=0.04), platelets ($\beta=0.054$, SE=0.021, p=0.02) and % sputum PMN ($\beta=0.263$, SE=0.126, p=0.05).

DISCUSSION

As endotoxin induces host innate immune cells to generate reactive oxygen species, we hypothesised that the GSTM1 null genotype would be associated with increased inflammatory response to inhaled endotoxin. When considered as a single cohort of 35 volunteers, these subjects had increased sputum and systemic PMNs 4 h following endotoxin (CCRE) challenge, without exhibiting any change in lung function, malaise or fever. These results are similar to those reported by our group and others¹⁰ using this level of endotoxin.

With stratification of the group on the basis of GSTM1 genotype, we found that 17 GSTM1 null volunteers had a significant increase in PMNs/mg sputum following inhalation of

CCRE, whereas 18 GSTM1 sufficient volunteers showed no significant change in PMNs/mg sputum following CCRE challenge. However, when differential counts of sputum cells are considered, both groups do have a significant increase in % sputum PMNs. Sputum IL-1 and TNF are also increased in both groups after challenge, suggesting that airway inflammatory responses, although muted in the GSTM1 sufficient group, are induced by inhaled CCRE in both GSTM1 genotypes. We also found that while both the GSTM1 null and sufficient groups had systemic PMN and WBC responses to CCRE, the GSTM1 null volunteers had a larger increase in circulating PMNs and WBCs, as well as an increase in platelets not observed in GSTM1 sufficient volunteers.

We employed linear regression analysis to further assess the role of the GSTM1 genotype in modifying the response to CCRE. Because we had previously reported that BMI correlated with airway neutrophilic response to CCRE,¹¹ and others have found that obesity is also a risk factor for adverse effects associated with ambient air PM exposure,⁷ we adjusted for BMI as well as ethnicity, gender, age, baseline levels of analytes, and GSTM1 genotype. Our linear regression analysis revealed that the GSTM1 null genotype was a significant modifier of the 4 h response of circulating WBCs, platelets and sputum PMNs (% sputum PMNs) to CCRE challenge. Overall, our results indicate that while those with the GSTM1 sufficient or null genotype have systemic and respiratory inflammatory response to inhaled endotoxin, this response is exaggerated in GSTM1 null volunteers compared to those who are GSTM1 sufficient.

Our observations are consistent with other studies in which GSTM1 null subjects have increased response to other pro-oxidant pollutants. In addition to the epidemiological studies outlined in the introduction, GSTM1 null volunteers with asthma have increased nasal response to allergen after experimental diesel exhaust and tobacco smoke exposure,⁶ and we have shown that those with the GSTM1 null genotype have increased PMN influx to the airway 24 h following exposure to 0.4 ppm O₃.⁹ In a cohort of volunteers who were exposed to 0.4 ppm O₃ and 20 000 EU CCRE on separate occasions, we found a strong within-individual correlation between O₃ and CCRE-evoked PMN response.¹² Thus, susceptibility to O₃ also correlated with susceptibility to endotoxin (CCRE). While this initial study is limited by a relatively small sample size, these results suggest that compared to the GSTM1 sufficient genotype, the GSTM1 null genotype confers greater risk for adverse health effects associated with exposure to a wide variety of environmental pollutants, including environmental endotoxin found in bioaerosols, airborne PM and second-hand tobacco smoke.

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What this paper adds

- ▶ Persons with the GSTM1 null genotype have increased respiratory and systemic inflammatory responses to experimental inhalational challenge to low dose endotoxin.
- ▶ As GSTM1 is an antioxidant enzyme, activation of oxidative responses in innate immune cells by endotoxin may play a role in mediating subsequent inflammatory responses to this agent.
- ▶ These results suggest that the GSTM1 null genotype should be considered a risk factor for adverse health effects associated with exposure to a wide variety of environmental pollutants, including environmental endotoxin found in bioaerosols, airborne particulate matter and second-hand tobacco smoke.

Figure 1.

Baseline versus post-CCRE (endotoxin) responses (mean $\pm$ SEM) of (A) circulating white blood cells (WBCs), (B) polymorphonuclear neutrophils (PMNs), (C) % PMNs in sputum and (D) sputum PMNs/mg sputum in GSTM1 sufficient and GSTM1 null volunteers.