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The Glutathione-S-Transferase null genotype and increased neutrophil response to low level ozone (0.06 ppm)

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To the Editor

We have previously reported that the GSTM1 null genotype is associated with increased inflammatory response to inhaled ozone (O_3) [1] and that O_3 modifies markers of innate immunity and inflammation in healthy individuals. [234] Although it is unclear what inflammatory risk factors account for increased susceptibility to O_3, the neutrophil (PMN) response to O_3 is a hallmark inflammatory marker that varies in magnitude across individuals – hence it has been used to identify O_3 responsive and non-responsive individuals in terms of inflammatory responsiveness. [563] We hypothesized that PMN responsive individuals would have modified cell responses and airway cytokine levels, and that genetic factors would modulate risk of PMN response to O_3.

In a recently published study by our group, 24 healthy adults (age 20-33 years) were examined 18 hours after a 6.6 hour controlled exposure to 0.06 ppm O_3 or clean air (CA). [7] That study revealed a significant O_3-indcued decrement in FEV_1 and an increase in neutrophilic inflammation in the airways. In this report we provide new measurements from that study including genotyping for genes reported to impact risk for response to O_3 (GSTM1, NQO1, TNF), sputum cell assessment of markers of innate immune activation and function and inflammatory cells and cytokines. Moreover, in this analysis we stratified the 24 subjects according to their PMN response to O_3 defined as, %PMN O_3- %PMN CA, such that PMN responsive individuals, or responders, were those who had %PMN O_3 - %PMN CA ≥ 10ten (N=13) versus those who had %PMN O_3 - %PMN CA < 10 (non-responders, N=11). Specifically, we examined by flow cytometry (BD LSR-II flow cytometer with BD FACSDiva 6.1 software, BD Biosciences Immunocytometry Systems, San Jose, CA) phenotype responses known to be modified by O3 such as cell surface marker expression

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(mean fluorescence intensity/MFI) of innate immune receptors (CD11b, CD14, CD16, CD86, HLA-DR, CD54), phagocytosis (% of cells undergoing phagocytosis of IgG opsonized zymosan A bioparticles), and inflammatory cytokines (IL-8, IL1β, IL-6, HA, TNFα) as measured by Meso Scale Discovery/MSD (Gaithersburg, MD) [24]. All flow cytometric endpoints and markers of inflammation were analyzed using non-parametric paired T-Tests comparing post- O_3 to post CA exposure time points. Regression analysis was used to determine the association between genotype status and PMN responsiveness. The following regression model was used: $logit(P(Responder)) = \alpha_0 + \alpha_1 Age + \alpha_2 I(Female) + \alpha_3 BSA + \alpha_4 BMI + \alpha_5 I(GSTM1-)$ where BSA = body surface area and BMI = body mass index. Statistical significance was determined at $p<0.05$ level for all analyses.

We observe that the mean (± SEM) PMN response to O_3 was significantly elevated in responders versus non-responders (27 ± 3 vs 3 ± 2, $p<0.05$) and that the mean (±SEM) FEV_1 response (% change from CA) was not significantly different between subject cohorts (-1.23% ± 0.8 vs -2.41% ± 2.1). Logistic regression analysis showed that responder status was strongly associated with GSTM1 status. The GSTM1 null genotype significantly ($p<0.05$) increased the probability of being a responder such that the calculated odds ratio of being a responder for the GSTM1 null genotype was estimated to be 13 (95%CI: 1.071 to 157.8). NQO1 and TNFa genotypes did not have a significant association with PMN response in this cohort. Post CA, responders had significantly ($p<0.05$) elevated levels of pro-inflammatory cytokines IL-8, IL-6 and TNFα compared to non-responders, and along with Hyaluronic Acid (HA), which has been implicated in inflammatory response to O_3, [89] these cytokines remained significantly elevated post O_3 when compared to post O_3 levels in non-responders (Table 1). Only IL-8 was significantly elevated post O_3 versus post CA in both responders and non-responders (Table 1). Sputum macrophage phagocytosis was also significantly elevated post CA in responders versus non-responders (51% ± 2 vs 45% ± 3, $p<0.05$), that together with the inflammatory cytokine data, suggests that PMN responders may have primed immuno-inflammatory features under non O_3 exposed conditions. We found that O_3 significantly enhanced the expression of immune cell surface receptors on macrophages (CD11b, CD14, CD16) and monocytes (HLA-DR, CD86, CD54) in responders but not in non-responders (Figure 1).

In summary, we report that individuals with an elevated PMN response to low level O_3 are 13 times more likely of having the GSTM1null genotype than non-responders. Furthermore, responders have increased immuno-inflammatory responses to O_3 compared to non-responders, and have elevated markers of inflammation following CA, suggesting the presence of a primed inflammatory airway in non-O3 exposed conditions. PMN responsiveness was also confirmed to be independent of the spirometric (FEV1) response to low level O_3 in healthy people. Since GSTM1 is a risk factor for asthma exacerbation and ozone, these data support the hypothesis that genetic modifiers of oxidative stress modulate the health effects of O_3 in individuals with allergic airways disease.

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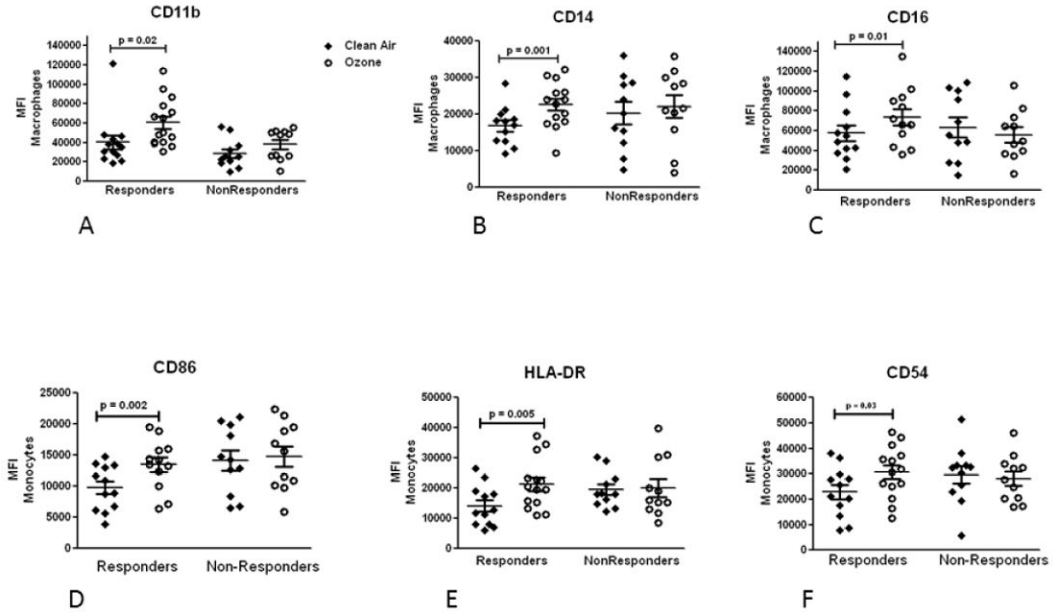

Figure 1.

Mean (±SEM) cell surface phenotype expression (MFI) in Responders and NonResponders following clean air (solid diamond) and ozone (open circle) exposure on sputum macrophages (A-C) and monocytes (D-F). Responders, but not NonResponders, had significantly ($p<0.05$) elevated expression for all markers after O3 versus clean air exposure. MFI: mean fluorescence intensity.

Table 1

Mean (SEM) Cytokine Levels Following Clean Air and Ozone Exposure

	Responders		Non-Responders	
Cytokine (pg/ml)	**Clean Air**	**Ozone**	**Clean Air**	**Ozone**
IL-8	6465 (1618) *#	9794 (2241) +	2772 (572) *	4441 (831)
IL-6	137 (53) #	134 (32) +	39 (10)	57 (11)
IL-1β	190 (68)	171 (37)	67 (13)	101 (28)
TNFα	15 (5) #	16 (4)	4 (1)	9 (3)
HA	11,737 (1428)	18,019 (5615) +	9064 (1474)	6976 (1522)

* p<0.05 Clean Air vs Ozone;

# p<0.05 Clean Air vs Clean Air;

+ p<0.05 Ozone vs Ozone;

HA hyaluronic Acid; pg/ml picograms per milliliter