Toxicology and Industrial Health 2002; **18:** 343–352
www.tihjournal.com

Dose-dependent thiol and immune responses to ovalbumin challenge in Brown Norway rats

Nabil H Al-Humadi[a,b], Joseph KH Ma[b], Daniel M Lewis[a], Jane YC Ma[a], Mark W Barger[a] and Paul D Siegel[a]

[a] *HELD, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA*
[b] *School of Pharmacy, West Virginia University, Morgantown, WV 26506, USA*

Dose-dependent specific antibody production, antigen-dependent pulmonary inflammation, and thiol changes in the lung and associated lymph nodes were examined in a Brown Norway rat model of pulmonary sensitization. Cysteine (CYSH), glutathione (GSH), and markers of inflammation in bronchoalveolar lavage fluid (BALF) were measured following ovalbumin (OVA) inhalation challenge. Alveolar macrophages (AM) and pulmonary-associated lymph node cells (LNC) were isolated and intracellular CYSH and GSH assessed. OVA-specific IgE and IgG antibodies were quantified from sera. A dose-dependent biphasic response was noted with respect to OVA-specific IgE. OVA-specific IgG concentrations were maximal at 68 mg (OVA)/m^3. OVA challenge to sensitized rats induced increases in BALF albumin, total protein, lactate dehydrogenase, CYSH and GSH that were independent of serum antibody concentrations. AM thiols were modestly elevated at low OVA challenge doses, but sharply reduced at the higher OVA challenge doses. In contrast, both thiols were dose dependently elevated in BALF. CYSH, but not GSH, was elevated in LNC of OVA challenged rats. In summary, antigen exposure caused a dose-dependent alteration of inflammatory, thiol and immune parameters in OVA sensitized and challenged rats. Changes in thiol levels did not correlate with antibody responses. While the results of the present study do not support a functional role for thiols in the immune response, it is important to note the dose-dependent dramatic alteration seen in thiols following sensitization and challenge. *Toxicology and Industrial Health* 2002; **18:** 343–352.

Key words: *alveolar macrophage; immunoglobulins; ovalbumin; parathymic and tracheal lymph nodes; thiols*

Introduction

In the last few decades, allergic disorders in affluent countries have steadily increased (Peat, 1994). In the food processing industry, workers may be exposed and sensitized to protein allergens. For example, workers at egg processing plants could be exposed for 8 h or more daily to ovalbumin aerosol concentrations as high as 360 μg/m^3 (National Institute for Occupational Safety and Health, 1988). The association between increased levels of specific antibodies (IgE and IgG) and exposure to ovalbumin in different animal models has been reported (Anderson, 1980; Sedgwick and Holt, 1983; McMenamin *et al*., 1992; Renz *et al*., 1992;

Address all correspondence to: Paul D Siegel, National Institute of Occupational Safety and Health, ASB, L4218, 1095 Willowdale Road, Morgantown, WV 26506-9530, USA
E-mail: pds3@cdc.gov

10.1191/0748233702th155oa

Siegel *et al*., 1997; 2000). Furthermore, ovalbumin has been used to investigate the effect of occupational and environmental factors on the development of respiratory and intestinal allergies (Watzl *et al*., 1999; Siegel *et al*., 2000; Al-Humadi *et al*., 2002).

Changes in the level of thiols are thought to play a role in immune responses (Droge *et al*., 1994). Glutathione (GSH), a major intracellular antioxidant, has an important protective function against oxidative cell damage (Wilhelm *et al*., 1997). The regulatory role of GSH in lymphocyte functions has been demonstrated in many studies (Fidelus *et al*., 1987; Suthanthiran *et al*., 1990; Potter *et al*., 1997). Furthermore, lymphocytes exhibit strong membrane transport activity for CYSH, and a relatively weak transport activity for cystine (Suthanthiran *et al*., 1990). In contrast, alveolar macrophages (AM) have a strong capacity for cystine uptake and the conversion of cystine into CYSH (Gmunder *et al*., 1990). In fact, Gmunder *et al.* (1990) reported that macrophages, when stimulated, function as a CYSH pump that takes up cystine and releases CYSH, resulting in an increase in the intracellular GSH level of activated lymphocytes in the vicinity. These studies suggest that macrophages may modulate T lymphocyte responses through thiol regulation.

Reactive oxygen species may also play an important role in immune responses (Oliveira *et al*., 1995; Wolfreys and Oliveira, 1997; Watzl *et al*., 1999). The imbalance between antioxidants, such as glutathione, and ROS levels may lead to oxidative stress and thus indirectly affect immune responses. It has been suggested that GSH, itself, may play a critical role in the immune changes. Macrophages, dendritic cells, and B cells (as antigen presenting cells) are central to the development of either Th1 or Th2 immunity through their function in antigen presentation and recognition (Peterson *et al*., 1998). Peterson *et al*. (1998) also reported that a selective inhibition of cytokine production in response to Th1-associated but not Th2-associated antigens, was due to GSH depletion in APC. Other studies demonstrated that the APC's ability to reduce antigen disulfide bonds required in antigen processing were impaired when GSH was severely depleted (Short *et al*., 1996). Thiol depletion may also decrease the activity of thiol proteases required in antigen processing and cleaving of the invariant chain of the major histocompatibility complex class II (Mizuochi *et al*., 1994). However, many factors other than the extracellular supply of thiols have been recognized as having an influence on the direction and magnitude of T cell-dependent responses, such as dose and route of antigen administration, type of APC, presence of particular hormones, co-stimulatory molecule expression, and peptide affinity for MHC determinants (Droge *et al*., 1994; O'Garra and Murphy, 1996; Constant and Bottomly, 1997). Lawrence *et al*. (2000) suggested that impairment of APC functions including antigen processing and presentation, as well as providing thiols necessary for complete T cell activation may be caused by the reduction of GSH levels.

The specific hypothesis of this research is that changes in thiol levels in the immune cells (due to antigen [OVA] exposure) might affect antibody production. Thus, we investigated the effect of allergic sensitization and challenge on thiol status in lymphocytes and alveolar macrophages and the potential links between the effect on this status and immune responses. In this study, we report the dose-dependent effect of OVA challenge in pulmonary sensitized BN rats specific IgE and IgG production and on intracellular CYSH and GSH in BALF, AM and LNC.

Materials and methods

Animal exposure

Male Brown Norway rats (Charles River, Stoneridge, NY), weighing 200–250 g, were used. Animals were housed in an American Association for Accreditation of Laboratory Animal Care (AAALAC)-approved facility and maintained at $23 \pm 1^{\circ}C$ with 50% relative humidity and a 12 hour light/dark cycle. Food and water were given *ad libitum.* Six different concentrations of OVA (Sigma Chemical, St Louis, MO) in endotoxin-free saline were aerosolized using a DeVilbiss-646 (DeVilbiss, Somerset, PA) nebulizer for OVA exposure. Infusion of the OVA solution, using a syringe pump (Model # 901, Harvard Apparatus, Dover, MA) at a flow rate of 1.22 mL/min to the nebulizer, maintained a constant fluid level in the nebulizer. OVA solutions of

1, 10, 12, 15, 30, and 35 mg/mL saline were used to obtain OVA chamber aerosol concentrations of 7.2, 68, 93, 111, 156 and 171 mg/m^3, respectively. This broad concentration range was used to be consistent with, and extend observations from our previous studies (Siegel *et al*., 2000; Al-Humadi *et al*., 2002) and ensure that dose dependency of the multiple parameters examined in this study could be evaluated. The aerosol size was measured using a GRIMM (Model # 1.108, Grimm Technologies, Douglasville, GA). Aerodynamic mass median diameter was 1.6 μm with standard geometric deviation, σ_g, of 1.44. The chamber OVA aerosol concentration was determined by collecting samples onto 0.45 μm (Polycarbonate Membrane, Poretics Corporation, Livermore, CA) filters from a chamber side port at a rate of 1 L/min. Filters were washed with 10 mL of endotoxin-free saline and analysed for protein using the Coomassie blue dye reagent (Bio-Rad Laboratories, Hercules, CA). Rats were exposed to OVA for 30 minutes at days 0, 7, 14, and 28. Control animals were exposed to aerosolized endotoxin-free saline.

Bronchoalveolar lavage (BAL) and biochemical assays

Rats were anaesthetized with sodium pentobarbital (50 mg/kg, ip; Butler, Columbus, OH) and euthanized by exsanguination one day after the last inhalation exposure (either day 1 or day 29). The trachea was cannulated and the lungs were lavaged with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS, 45 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, and 5.5 mM glucose; pH 7.4), at a volume of 6 mL for the first lavage and 8 mL for the subsequent lavages. The total lavage volume was 80 mL. The BAL fluid was centrifuged at 500 *g* for 10 minutes at 4°C. The supernatant from the first lavage was saved separately for the determination of lactate dehydrogenase (LDH) activity, protein and albumin levels using an automated Cobas Fara II analyser (Roche Diagnostic Systems, Montclair, NJ) with standard diagnostic reagents and manufacturers procedures. GSH and CYSH were also measured in the BALF fraction by the method described below. The cell pellet from all lavage fluids of an individual rat was suspended in 1 mL of PBS to determine the total cell and differential cell counts using an electronic cell counter (Coulter Electronics, Hialeah, FL) equipped with a cell-sizing unit (Lane and Mehta 1990). Alveolar macrophages and granulocytes were determined by their unique cell diameters.

LNC preparation

Lymph nodes (para-thymic and tracheal) were collected in 1 mL, pH 7.4, Hepes buffer. The lymph node capsules were broken with a glass bar using a tissue culture screen. LN cells were washed, transferred into a 15-mL tube, centrifuged at 2000 rpm for 10 min, and resuspended in 1 mL of Hepes buffer for cell counts, cell differentiation, and thiol determinations. The cell samples prepared showed a lymphocyte content of greater than 98%.

Cysteine and GSH determination in AM and lymphocytes

AM isolated from each rat were suspended in eagle minimum essential medium (EMEM, Sigma Chemical Co.) containing 1 mM glutamine, 100 μg/mL streptomycin, 100 units/mL penicillin, 10% heat-inactivated fetal bovine serum, and 10 mM Hepes. Aliquots of 1 mL cell suspension, adjusted to 1×10^6 AM, were added to each well of a 12-well tissue culture plate. AM were allowed to adhere to the plastic plate for two hours (for cell purification purpose) in a humidified incubator (37°C and 5% CO_2) (Driscoll *et al*., 1990). The nonadherent cells were removed by rinsing the monolayers three times with Hepes buffer. After centrifugation at 500 *g* for five minutes, the macrophage cell pellet was washed twice and reconstituted in 0.5 mL Hepes and analyzed for cysteine and GSH contents according to the following method. One microlitre of a solution containing 80 mM monobromobimane (mbb) was added to 100 μL of the cell suspension. The fluorescence derivatization of the reduced thiols was allowed to proceed at 4°C overnight. The cells were then lysed by sonication for 30 seconds using a sonic dismembrator (Fisher Scientific, Pittsburgh, PA), centrifuged at 10 000 rpm for 10 minutes to remove cell membranes and debris, and filtered through a 0.2 μm filter (Microspin Nylon Filter, PGC, Frederick, MD). Analysis of cysteine and GSH levels in the solution was carried out using a reverse phase high performance liquid chromatography (HPLC) method.

For lymphocytes, aliquots of 2×10^6 cells were used after washing (centrifuged at 500 *g* for five minutes) twice with Hepes. The pellets were resuspended in 1 mL Hepes buffer and analysed for cysteine and GSH following the method described above.

HPLC analysis

A Shimadzu HPLC system equipped with a RF-551 fluorometric detector and a C18 reverse phase column (Phenomenex, Luna, 5 μm) was used for the analysis of cysteine and GSH. Separation of the mbb-thiol derivatives was achieved using a gradient system of acetonitrile in 0.1 M sodium acetate (pH 5.0), delivered at a flow rate of 1 mL/min. The gradient system consisted of a mobile phase of 7% acetonitrile for the first six minutes, and stepped increases to 15, 25, and 40% after 10, 15, and 18 minutes, respectively. The cysteine and GSH derivatives were detected at an excitation of 380 nm and an emission of 480 nm, with retention times at 8.0 and 10.2 minutes, respectively.

Serum OVA specific IgE and IgG measurements

Blood samples were collected during exsanguination from the vena cava at sacrifice. Four sera dilutions with 5% horse serum albumin (HOSA)/PBS of 1/100, 1/500, 1/1000, and 1/10 000 were analysed for OVA-specific IgE. The OVA-specific IgG determinations were obtained on sera dilutions of 1/10 000, 1/50 000, 1/100 000, and 1/500 000. Diluted sera (100 μL/well) were added to a 96-well plate (ICN Biomedicals, Inc., Horsham, PA) that had been previously coated with 200 μL of 1% OVA carbonate coating buffer and blocked with a 5% HOSA/coating buffer according to the method of Voller and Bidwell (1986). The plates were incubated overnight at 4°C and subsequently incubated with sheep anti-rat IgE (100 μL/well, 1/5000 dilution in HOSA/PBS; Cat. No. 64-352, ICN Biomedicals, Inc., Costa Mesa, CA) and horseradish peroxidase-bound donkey anti-sheep IgG (100 μL/well, 1/10 000 dilution in HOSA/PBS; Cat. No. 67541, ICN Biomedicals, Inc., Costa Mesa, CA) for two hours each at room temperature. The plates were washed three times following each incubation, treated with tetramethylbenzidine (TMB, Sigma Chemical Co., Saint Louis, MO), and read at 570 nm. OVA-specific IgG were determined using Goat anti-rat IgG (1/1000 dilution in HOSA/PBS; Cat. No. R5005, Sigma Chemical Co., Saint Louis, MO) and peroxidase-labelled rabbit anti-goat IgG (1/25 000 dilution in HOSA/PBS; Cat. No. A-3540, Sigma Chemical Co., Saint Louis, MO) as detection antibodies following the same protocol described above. Pooled serum from OVA sensitized animals was extensively titered and concentration/response curves were obtained. These curves were then used as references to obtain relative concentrations for the OVA-specific IgE and IgG of different exposure groups. A relative concentration of 100 is equal to the amount of specific IgE or IgG in the neat (undiluted pooled sera) reference standard.

Data analysis

All data were expressed as mean ± standard error of two to four separate experiments. Each measurement was run in duplicate or triplicate per experiment. Statistical analysis of data was performed using Sigma Stat (version 2.0, Jandel Scientific Software, San Rafael, CA) statistical software for Windows 95, NT, and 3.1. ANOVA (Tukey's or Dunn's) or *t*-test was conducted and values of $P < 0.05$ were considered statistically significant. Relationships between parameters measured, independent of exposure dose, were examined by ranking the responses within each exposure group (1 to 5), then using the Spearman Rank Order Correlation test on the pooled rankings.

Results

The number of granulocytes, LDH activity, total protein content, and albumin levels from BAL fluid are shown in Figure 1. An OVA dose-dependent relationship was seen for LDH, total protein, and albumin. These results are consistent with leakage across both pulmonary epithelial and cellular membranes following antigen challenge to sensitized rats.

Figure 2 shows the dose-dependent relationship between OVA exposure and serum-specific antibody levels. Maximal serum concentrations of antigen-specific IgE and IgG in rats were noted at 68 mg/m^3 OVA. A trend of decreasing OVA-specific

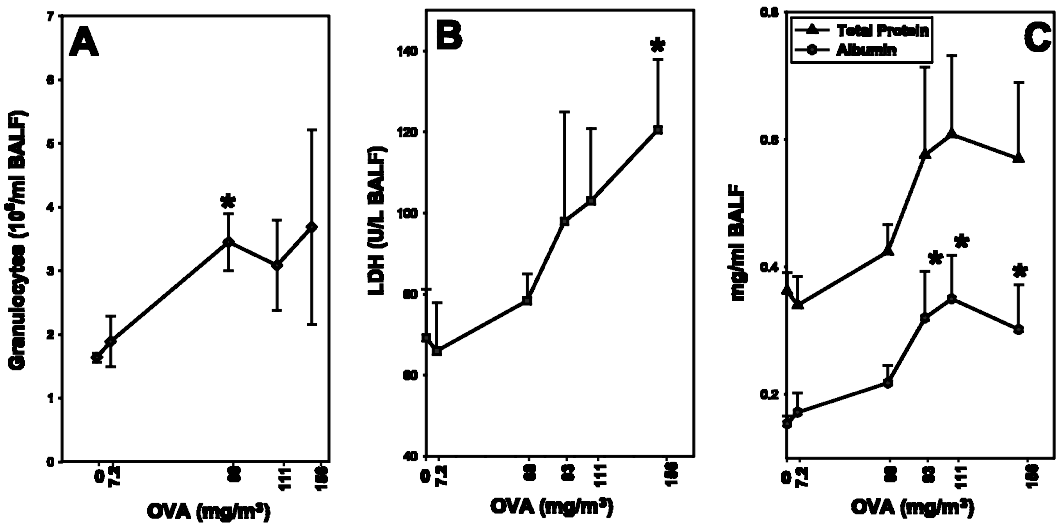

Figure 1. OVA dose-dependent inflammatory responses in sensitized Brown Norway rats following inhalation challenge as indicated by elevations of granulocytes (A), lactate dehydrogenase activity (B), total protein and albumin (C) content in bronchoalveolar lavage fluid (BALF). $n = 5$/group. Each point represents average$\pm$SEM on a log/linear scale. * Significantly different from saline control at $P < 0.05$, t-test.

IgE serum levels was seen at exposure doses $\geq$ 93 mg/m^3 OVA with a statistically significant decreased serum concentration noted at 156 mg/m^3.

The levels of cysteine and GSH were determined in bronchoalveolar lavage fluids (Figure 3). Both cysteine and GSH levels increased dose dependently resulting in an approximate 50-fold increase at the 156 mg/m^3 exposure level.

A significant increase in the cysteine level of both AM and LNC was noted after a single sensitization exposure to 171 mg/m^3 OVA in naïve (non-sensitized) rats (Figure 4A, C). There were no changes in GSH levels of these cells (Figure 4B, D).

Figure 5 shows the intracellular cysteine and GSH levels in AM from the various OVA sensitized and challenged groups. Cysteine at the lowest OVA exposure dose increased slightly followed by a

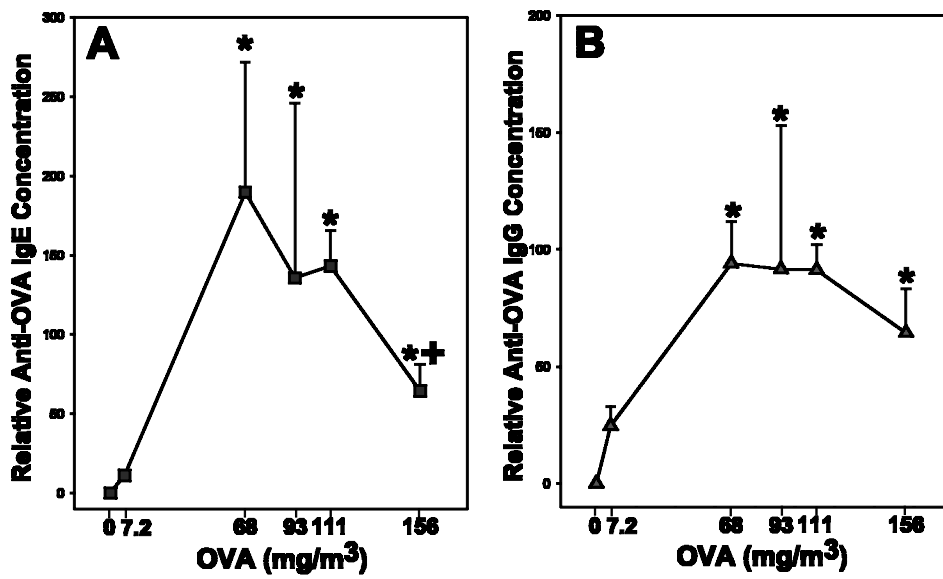

Figure 2. The relative serum anti-OVA IgE (A) and anti-OVA IgG (B) concentrations following repeated exposure to saline or various doses of OVA. $n = 5$/group. Each point represents average$\pm$SEM on a log/linear scale. * Significantly different from saline control at $P < 0.05$, t-test. + Significantly different from 68 mg/m^3 at $P < 0.05$, t-test.

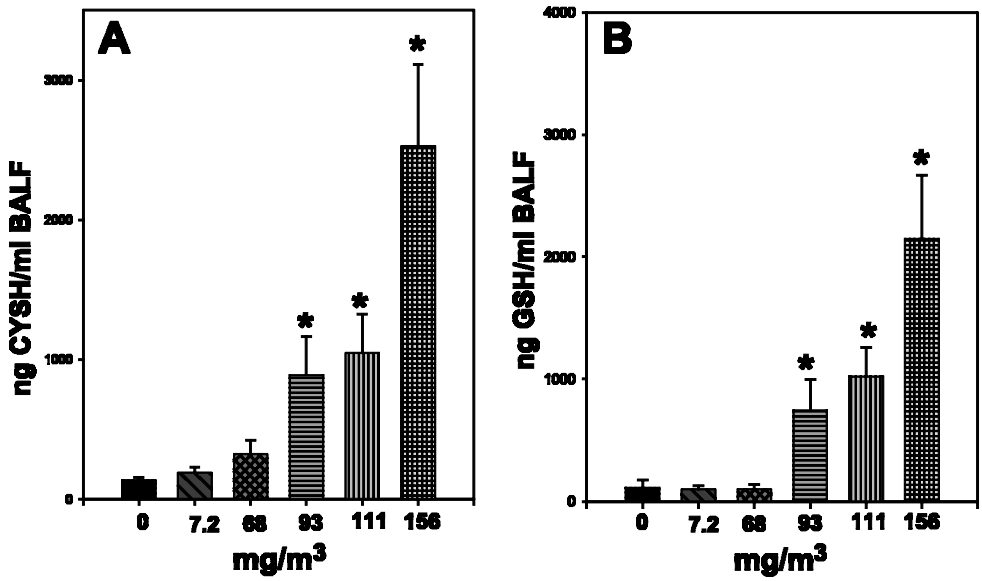

Figure 3. OVA dose-dependent cysteine (CYSH, A) and GSH (B) levels in bronchoalveolar lavage fluids (BALF) following inhalation challenge of sensitized rats. $n = 5$/group. Each point represents average±SEM. * Significantly different from saline control at $P < 0.05$, ANOVA.

sharp decrease at all the higher OVA exposure doses. Glutathione levels were increased at low doses and reached significance (1.25 times control) at 68 mg/m^3 dose. A marked decrease (1/10 of control) in GSH levels was seen at higher OVA exposure doses. Figure 6 shows the cysteine and glutathione levels in LNC from OVA sensitized and challenged rats. There was a significant increase in the cysteine levels at high OVA exposure doses. Glutathione levels in these cells were not changed.

Each of the exposure groups was challenged to the same aerosol concentration that was used for sensitization of that group. Each individual response was ranked (from 1 to 5) within each OVA exposure group and the Spearman Rank Order Correlation performed across all exposures to evaluate the relationships between specific antibodies, thiols and pulmonary inflammation. Poor (no) relationships were found between either specific IgE or IgG and any of the thiol measurements or

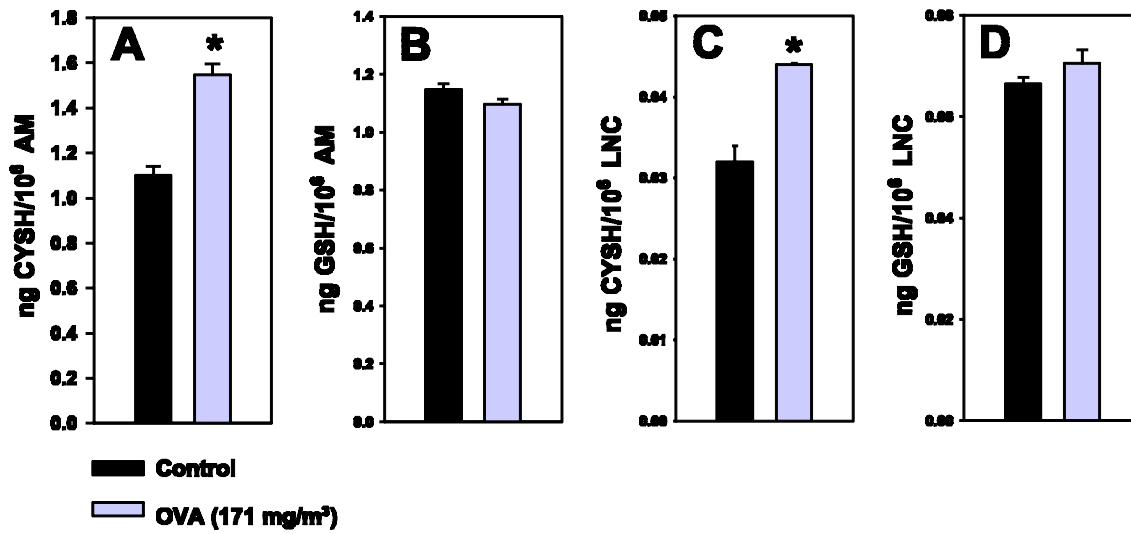

Figure 4. Effect of a single (171 mg/m^3) OVA-sensitization exposure on intracellular cysteine (CYSH) and glutathione (GSH) levels in alveolar macrophages (AM) (A and B respectively) and para-thymic and tracheal lymph node cells (contain >98% lymphocytes) (C and D respectively). $n = 5$/group. Each point represents average±SEM. * Significantly different from saline control at $P < 0.05$, ANOVA.

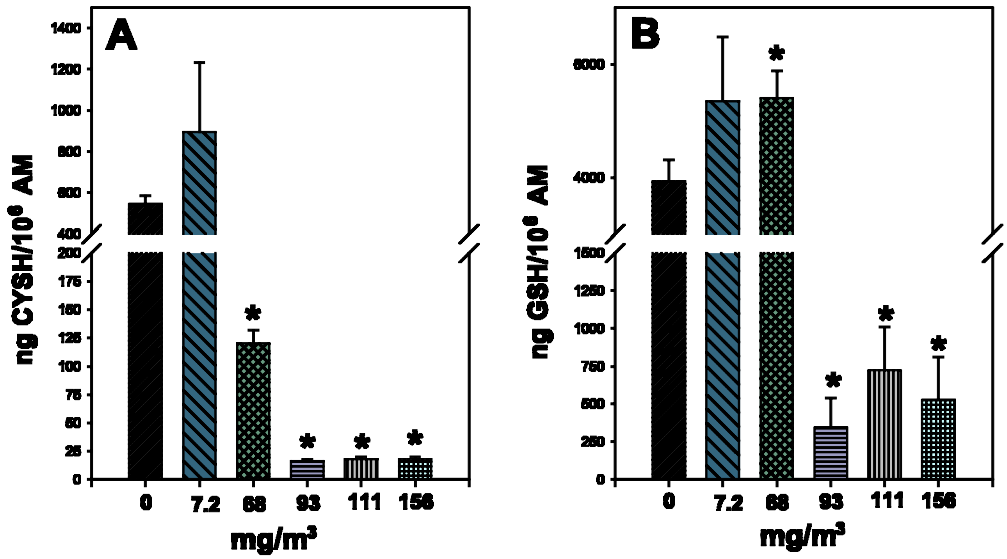

Figure 5. Dose-dependent changes following OVA challenge in intracellular cysteine (CYSH, A) and GSH (B) alveolar macrophages (AM) level from sensitized rats. $n = 5$/group. Each point represents average±SEM. * Significantly different from saline control at $P < 0.05$, ANOVA.

pulmonary inflammatory markers. High correlations were found between BALF LDH and BALF albumin ($r = 0.725$, $P < 0.001$); BALF LDH and BALF cysteine ($r = 0.78$, $P < 0.001$); BALF cysteine and BALF GSH ($r = 0.84$, $P < 0.001$); and AM cysteine and AM GSH ($r = 0.92$, $P < 0.001$). When looking only at a concentration of 93 mg/m^3 where high dose tolerance was noted for specific IgE, a strong relationship between specific IgG and pulmonary inflammation (BALF LDH and albumin) was noted ($r = 0.733$, $P = 0.00145$ for both). In addition, BALF LDH and AM cysteine were inversely related ($r = -0.800$, $P < 0.001$).

Discussion

The Brown Norway rat model of pulmonary allergic sensitization was used in this study to examine the potential dose-dependent relationships between specific antibody serum concentrations, pulmonary inflammation, and thiol levels in BALF,

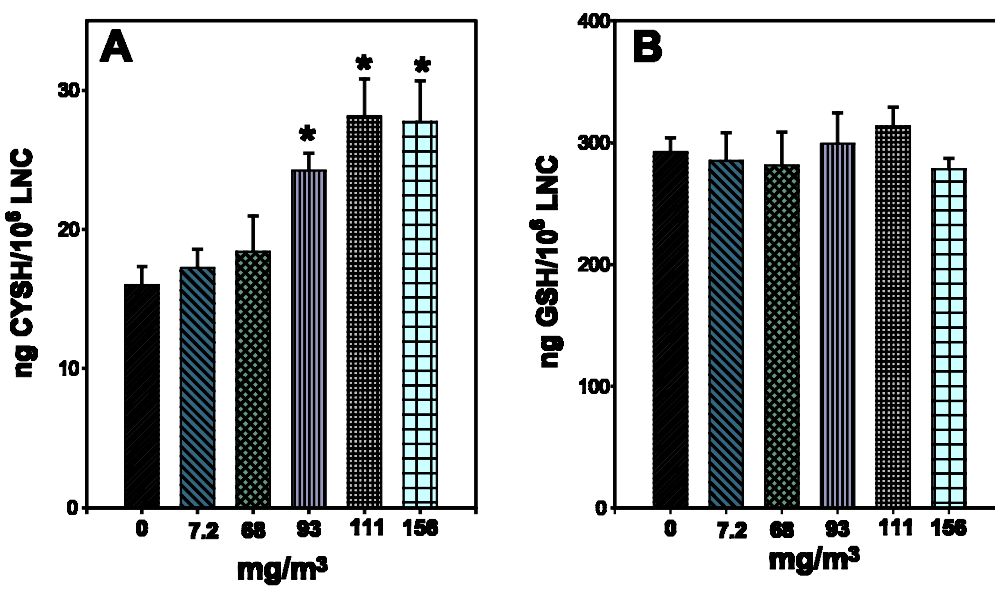

Figure 6. Dose-dependent changes following OVA challenge in intracellular cysteine (CYSH, A) and GSH (B) in para-thymic and tracheal lymph node cells from sensitized rats. $n = 5$/group. Each point represents average±SEM. * Significantly different from saline control at $P < 0.05$, ANOVA.

AM and LNC. Immune response changes due to antigen (such as OVA) exposure have been connected to thiol changes in the cell and its surrounding environment. A direct relationship between T cell GSH levels and T cell function has been reported in GSH depletion experiments. Suppression of T cell activation (Droge *et al*., 1991; Kavanagh *et al*., 1993; Staal *et al*., 1994; Otsuji *et al*., 1996; Potter *et al*., 1997; Wilhelm *et al*., 1997), proliferation (Fidelus *et al*., 1987; Gmunder *et al*., 1990; Suthanthiran *et al*., 1990; Smyth, 1991; Walsh *et al*., 1995; Taylor *et al*., 1997), IL-2R expression (Liang *et al*., 1992), cytokine production (Peterson *et al*., 1998; Lawrence *et al*., 2000), and generation of CTL responses (Gmunder and Droge, 1991; Multhoff *et al*., 1995) by GSH depletion have been reported.

OVA-specific IgE sera levels were dose dependent. The IgE response was biphasic, with the decrease in specific IgE at the higher doses (Figure 2). The relationship between thiol and immune regulation may be very complex. As noted with the alveolar macrophage, one cell type may become dysfunctional with respect to its thiol regulation, even while its surroundings are thiol rich. Allergic sensitization is a multifactorial process involving many cell types. It cannot be determined if the AM reflects all APC's or if it is atypical since they exist outside the body in alveolar spaces and lack APC accessory molecules. The role of AM as an APC remains controversial.

The early sensitization responses to single high acute OVA exposure dose (171 mg/m^3) were studied (Figure 4). This response was in agreement with our previous study (Al-Humadi *et al*., 2002). Acute exposure alone caused a significant increase in the levels of cysteine and no changes in the GSH levels. The antigen, OVA, may be one possible source of cysteine upon catabolism by the cells. These data suggest that exposure to high concentrations of antigen are not responsible for the severe depletion of cellular thiols observed upon OVA challenge to pulmonary sensitized rats. The loss of reduced thiol is most likely due to production of free radicals during the allergic response to antigen challenge.

Severe depletion in both CYSH and GSH levels in AM (Figure 5) from rats sensitized and challenged with 93 mg/m^3 was observed. This depletion was concomitant with the beginning of the high exposure dose-dependent trend of decreased circulating specific-IgE (Figure 2). However, there was no relationship between AM thiols and specific IgE serum concentrations independent of sensitization/challenge dose (Spearman Rank Order Correlation for specific IgE versus AM GSH and cysteine: and $r = -0.04$, $P = 0.847$; $r = 0.02$, $P = 0.922$, respectively). The reason for the loss of thiols is not known. Loss could possibly come from oxidative stress, catabolism for the production of thiol containing peptides/proteins or potentially the monocyte population in the alveoli may have changed with respect to maturity or phenotype of cells.

It is possible that different mechanisms were involved at the higher exposure doses (≥ 93 mg/m^3) where IgE tolerance was suggested, so the analyses were repeated using only those groups. Again, no association was found between specific IgE and either inflammatory or thiol parameters, but there was a strong inverse relationship between BALF LDH, a marker of cellular damage, and AM cysteine content ($P < 0.001$). There were also fairly good correlations between BALF LDH vs AM GSH and BALF cysteine, but clustering of the data in a small range weakens any conclusions that may be drawn concerning these individual parameters. The relationships noted, however, do suggest that the thiol changes observed in lung tissues, at least in part, were related to the acute pulmonary inflammatory response to antigen in sensitized rats. In addition, our previous work demonstrated that OVA exposures to 90 mg/m^3 impaired the ability of AM to take up cysteine, reduce it and use it as a source of thiols (Al-Humadi *et al*., 2002). Together these results suggest that AM were not the predominant source of the BALF thiols.

The cysteine in LNC increased with increasing OVA exposure concentration (Figure 6). This may be due to several factors. Lymphatic drainage from the lung was probably rich in thiols as suggested by the high cysteine and GSH BALF levels. Ovalbumin, itself, is a cysteine-rich protein that may have contributed to the overall cysteine pool. LNC are lymphocytes composed primarily of B-cells and the various types of T-cells at varying stages of maturity. It is probable that the composition of lymphocytes comprising LNC was quite different between control and OVA sensitized/challenged rats

(although phenotyping of this population was not done in the present study) and that this contributed to the observed difference in cysteine levels. Even though cysteine levels were significantly increased in LNC, our previous results demonstrated that the ability of these cells to take up cystine and reduce it to cysteine (*ex vivo*) after a similar exposure regimen to 90 mg/m^3 OVA is greatly reduced (Al-Humadi *et al*., 2002).

Specific IgG, at the higher doses (93 mg/m^3) was strongly associated with the pulmonary inflammatory parameters (IgG vs BALF LDH and BALF albumin: $r = 0.733$, $P = 0.00145$ for both). The pulmonary inflammation observed at high OVA challenge exposure may have been the result of an immune complex, Arthus type of reaction. The relatively weak pulmonary cellular inflammatory response as measured in BALF from the BN rat model was not surprising. Our previous studies using this model found that BALF inflammatory cell evaluation was an insensitive tool for assessing pulmonary allergic inflammation (Siegel *et al*., 1997) and that the eosinophilic inflammation observed was primarily in the submucosa and lamina propria regions of the airway (Siegel *et al*., 2000). Biochemical measures of inflammation were found to be more sensitive measures of immune-mediated pulmonary inflammation in the present study. The strong association of specific IgG (at higher OVA exposure doses) together with the lack of association of specific IgE with the biochemical pulmonary inflammatory parameters points out that immune-mediated pulmonary inflammation observed following high dose antigen challenge is most likely associated with IgG.

In summary, this study showed a dose-dependent response to OVA-specific IgE and IgG and this response was biphasic for IgE. The increases in BALF albumin, total protein, lactate dehydrogenase, CYSH and GSH induced by OVA challenge to sensitized rats were independent of serum antibody concentration. A different pattern of CYSH and GSH changes were seen in BALF, AM and LNC in response to OVA exposure. While alteration in thiols has been reported in the literature to influence the nature of immune responses, the potential mechanistic role that they may have in the development and/or modulation of these responses has yet to be demonstrated.

Acknowledgements

Supported in part by NIH grant HL 62630-02.

References

Al-Humadi, N.H., Siegel, P.D., Lewis, D.M., Barger, M.W., Ma, J.Y.C., Weissman, D.N. and Ma, J.K.H. 2002: The effect of diesel exhaust particles (DEP) and carbon black (CB) on thiol changes in pulmonary ovalbumin allergic sensitized brown Norway rats. *Experimental Lung Research* 28, 333–49.

Anderson, P. 1980: Antigen-induced bronchial anaphylaxis in actively sensitized guinea pigs. *Allergy* 35, 61–71.

Constant, S.L. and Bottomly, K. 1997: Induction of Th1 and Th2 CD+4 T cell responses: The alternative approaches. *Annual Review of Immunology* 15, 297–322.

Driscoll, K.E., Lindenschmidt, R.C., Maurer, J.K. and Ridder, G. 1990: Pulmonary response to silica or titanium dioxide: inflammatory cells, alveolar macrophage-derived cytokines, and histopathology. *American Journal of Respiratory Cell and Molecular Biology* 2, 381–90.

Droge, W., Eck, H., Gmunder, H. and Hihm, S. 1991: Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives. *The American Journal of Medicine* 91, 1405–45.

Droge, W., Schulze-Osthoff, K., Mihm, S., Galter, D., Schenk, H., Eck, H., Roth, S. and Gmunder, H. 1994: Functions of glutathione and glutathione disulfide in immunology and immunopathology. *The FASEB Journal* 8, 1131–38.

Fidelus, R., Ginouves, P., Lawrence, D. and Tsan, M. 1987: Modulation of intracellular glutathione concentrations alters lymphocyte activation and proliferation. *Experimental Cell Research* 170, 269–75.

Gmunder, H. and Droge, W. 1991: Differential effects of glutathione depletion on T cell subsets. *Cellular Immunology* 138, 229–37.

Gmunder, H., Eck, H.P., Benninghoff, Roth, S. and Droge, W. 1990: Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine. *Cellular Immunology* 129, 32–46.

Kavanagh, T., Grossmann, A., Jinneman, J., Kanner, S., White, C., Eaton, D., Ledbetter, J. and Rabinovitch, P. 1993: The effect of 1-chloro-2,4-dinitrobenzene exposure on antigen receptor (CD3)-stimulated transmembrane signal transduction in purified subsets of human peripheral blood lymphocytes. *Toxicology and Applied Pharmacology* 119, 91–99.

Lane, F.C. and Mehta, J.R. 1990: *In vitro* human tumor sensitivity assay using cell counting and sizing. *American Biotechnology Laboratory* 8, 12–27.

Lawrence, B.P., Will, Y., Reed, D.J. and Kerkvliet, N.I. 2000: γ-Glutamyltranspeptidase knockout mice as a model for understanding the consequences of diminished glutathione on T cell-dependent immune responses. *European Journal of Immunology* 30, 1902–10.

Liang, S., Lee, N., Chen, Y. and Liang, C. 1992: Effects of glutathione on the synthesis and turnover of interleukin-2 receptors. *Cellular Immunology* 144, 131–42.

McMenamin, C., Girn, B. and Holt, P.G. 1992: The distribution of IgE plasma cells in lymphoid and non-lymphoid tissues in high-IgE responder rats: different localization of antigen-specific and 'bystander' components of the IgE response to inhaled antigen. *Immunology* 77, 592–96.

Mizuochi, T., Yee, S.T., Kasai, M., Kakiuchi, T., Muno, D. and Kominami, E. 1994: Both cathepsin B and cathepsin D are necessary for processing of ovalbumin as well as for degradation of class II MHC invariant chain. *Immunology Letters* 43, 189–93.

Multhoff, G., Meier, T., Botzler, C., Wiesnet, M., Allenbacher, A., Wilmanns, W. and Issels, R. 1995: Differential effects of ifosfamide on the capacity of cytotoxic T lymphocytes and natural killer cells to lyse their target cells correlate with intracellular glutathione levels. *Blood* 85, 2124–31.

National Institute for Occupational Safety and Health. 1988: Health Hazard Evaluation. *Report HETA* 86. Cincinnati, OH, 447–1919.

O'Garra, A. and Murphy, K. 1996: Role of cytokines in development of Th1 and Th2 cells. In Romangnani, S., editor, *Chemical immunology: Th1 and Th2 cells in health and disease*. Karger and Basel.

Oliveira, D.B.G., Gillespie, K., Wolfreys, K., Mathieson, P.W., Qasim, F. and Coleman, J.W. 1995: Compounds that induce autoimmunity in the Brown Norway rat sensitize mast cells for mediator release and interleukin-4 expression. *European Journal of Immunology* 25, 2259–64.

Otsuji, M., Kimura, Y., Aoe, T., Okamoto, Y. and Saito, T. 1996: Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 zeta chain of T-cell receptor complex and antigen-specific T-cell responses. *Proceedings of the National Academy of Sciences of the USA* 93, 13119–24.

Peat, J.K. 1994: The rising trend in allergic illness: which environmental factors are important? *Allergy* 24, 797–800.

Peterson, J.D., Herzenberg, L.A., Vasquez, K. and Waltenbaugh, C. 1998: Glutathione levels in antigen presenting cells modulate Th1 versus Th2 response patterns. *Proceedings of the National Academy of Sciences of the USA* 95, 3071–76.

Potter, A., Grossmann, A., Rabinovitch, P., Eaton, D. and Kavanagh, T. 1997: The effect of *in vitro* phorone exposure on glutathione content of T cell antigen receptor (CD3)-stimulated calcium mobilization in murine splenic T lymphocytes. *Toxicology in vitro* 11, 355–63.

Renz, H., Smith, H.R., Jenson, J.E., Ray, B.S., Irvin, C.G. and Gelfand, E.W. 1992: Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *The Journal of Allergy and Clinical Immunology* 89, 1127–38.

Sedgwick, J.D. and Holt, P.G. 1983: Induction of IgE-isotype specific tolerance by passive antigenic stimulation of the respiratory mucosa. *Immunology* 50, 625–30.

Short, S., Merkel, B.J., Caffrey, R. and McCoy, K.L. 1996: Defective antigen processing correlates with a low level of intracellular glutathione. *European Journal of Immunology* 26, 3015–20.

Siegel, P.D., Al-Humadi, N.H., Millecchia, L.L., Robinson, V.A., Hubbs, A.F., Nelson, E.R. and Fedan, J.S. 2000: Ovalbumin aeroallergen exposure-response in Brown Norway rats. *Inhalation Toxicology* 12, 245–61.

Siegel, P.D., Al-Humadi, N.H., Nelson, E.R., Lewis, D.M. and Hubbs, A.F. 1997: Adjuvant effect of respiratory irritation on pulmonary allergic sensitization: time and site dependency. *Toxicology and Applied Pharmacology* 144, 356–62.

Smyth, M. 1991: Glutathione modulates activation-dependent proliferation of human peripheral blood lymphocyte population without regulating their activated function. *Journal of Immunology* 146, 1921–27.

Staal, F., Anderson, M., Staal, G., Herzenberg, L., Gitler, C. and Herzenberg, L. 1994: Redox regulation of signal transduction: tyrosine phosphorylation and calcium influx. *Proceedings of the National Academy of Sciences of the USA* 91, 3619–22.

Suthanthiran, M., Anderson, M., Sharma, V. and Meister, A. 1990: Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. *Proceedings of the National Academy of Sciences of the USA* 87, 3343–47.

Taylor, C.G., Potter, A.J. and Rabinovitch, P.S. 1997: Splenocyte glutathione and CD3-mediated cell proliferation are reduced in mice fed a protein-deficient diet. *The Journal of Nutrition* 127, 44–50.

Voller, A. and Bidwell, D. 1986: Enzyme-linked immunosorbent assay. In Rose, N.R., Friedman, H. and Fahey, J.L., editors, *Manual of clinical laboratory immunology*, third edition. Washington, DC: American Society for Microbiology.

Walsh, A., Michaud, S., Malossi, J. and Lawrence, D. 1995: Glutathione depletion in human T lymphocytes: analysis of activation associated gene expression and the stress response. *Toxicology and Applied Pharmacology* 133, 249–61.

Watzl, B., Abrahamse, S.L., Treptow-Van Lishaut, S., Neudecker, C., Hansch, G.M., Rechkemmer, G. and Pool-Zobel, B.L. 1999: Enhancement of ovalbumin-induced antibody production and mucosal mast cell response by mercury. *Food and Chemical Toxicology* 37, 627–37.

Wilhelm, D., Bender, K., Knebel, A. and Angel, P. 1997: The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including jun N-terminal protein kinases and p38 kinase by alkylating agents. *Molecular and Cellular Biology* 17, 4792–800.

Wolfreys, K. and Oliveira, D.B.G. 1997: Alterations in intracellular reactive oxygen species generation and redox potential modulate mast cell function. *European Journal of Immunology* 27, 297–306.