

Quantum dots and mouse strain influence house dust mite-induced allergic airway disease

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ABSTRACT

Quantum dot nanoparticles (QDs) are engineered nanomaterials (ENMs) that have utility in many industries due to unique optical properties not available in small molecules or bulk materials. QD-induced acute lung inflammation and toxicity in rodent models raise concerns about potential human health risks. Recent studies have also shown that some ENMs can exacerbate allergic airway disease (AAD).

In this study, C57BL/6J and A/J mice were exposed to saline, house dust mite (HDM), or a combination of HDM and QDs on day 1 of the sensitization protocol. Mice were then challenged on days 8, 9 and 10 with HDM or saline only.

Significant differences in cellular and molecular markers of AAD induced by both HDM and HDM + QD were observed between C57BL/6J and A/J mice. Among A/J mice, HDM + QD co-exposure, but not HDM exposure alone, significantly increased levels of bronchoalveolar lavage fluid (BALF). IL-33 compared to saline controls. BALF total protein levels in both mouse strains were also only significantly increased by HDM + QD co-exposure. In addition, A/J mice had significantly more lung type 2 innate lymphoid cells (ILC2s) cells than C57BL/6J mice. A/J lung ILC2s were inversely correlated with lung glutathione and MHC-II^{high} resident macrophages, and positively correlated with MHC-II^{low} resident macrophages.

The results from this study suggest that 1) QDs influence HDM-induced AAD by potentiating and/or enhancing select cytokine production; 2) that genetic background modulates the impact of QDs on HDM sensitization; and 3) that potential ILC2 contributions to HDM induced AAD are also likely to be modulated by genetic background.

1. Introduction

Quantum dot nanoparticles (QDs) are engineered nanomaterials (ENMs) with intriguing optical properties including broad absorption profiles, sharp emission spectra, and excellent photostability (Medintz et al., 2005). These unique properties have enable a number of promising applications in biomedical research, medicine, and electronics

industries (Wu et al., 2003; Kim et al., 2004; Hu et al., 2010; Jung et al., 2012). However, QDs and other ENMs have been shown to cause lung inflammation and toxicity in mice and rats following pulmonary exposures by inhalation and other surrogate methods including intranasal and intratracheal instillation and oropharyngeal aspiration (Ma-Hock et al., 2009; Wang et al., 2011; Ma-Hock et al., 2012; McConnachie et al., 2013; Roberts et al., 2013; Scoville et al., 2015; Seiffert et al.,

Abbreviations: AAD, Allergic airway disease; AHR, Airway hyperresponsiveness; Rn, Airway Resistance; BALF, Bronchoalveolar lavage fluid; C, Compliance; ENMs, Engineered nanomaterials; E, Elastance; FBS, Fetal bovine serum; FMO, Fluorescence minus one; GSH, Glutathione; HDM, House dust mite; HBSS, Hank's balanced salt solution; IFN- γ , Interferon gamma; IL-4, Interleukin 4; IL-5, Interleukin 5; IL-6, Interleukin 6; IL-10, Interleukin 10; IL-13, Interleukin 13; IL-17A, Interleukin 17A; IL-33, Interleukin 33; KC, Keratinocyte chemoattractant; LOD, Lower limit of detection; MTCH, Methacholine; OVA, Ovalbumin; OPA, Oropharyngeal aspiration; PBS, Phosphate buffered saline; QDs, Quantum dot nanoparticles; R, Resistance; ILC2, Type 2 innate lymphoid cells; Th2, Type 2 T-helper cell; Th1, Type 1 T-helper cell; TOPO-PMAT, Tri-n-octylphosphine oxide poly(maleic anhydride-alt-1-tetradecene); G, Tissue Damping; H, Tissue Elastance

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2015; Silva et al., 2015; Cartwright et al., 2016). There is also emerging evidence that occupational exposures to ENMs may increase the risk of adverse respiratory and cardiovascular outcomes in humans (Cui, 2013; Liao et al., 2014).

Currently, approximately 8% of the US population is estimated to suffer from asthma, which is commonly characterized by chronic type 2 T-helper (Th2) cell associated airway inflammation and hyperresponsiveness (Kim and Mazza, 2011; CDC, 2017). Increased eosinophils and Th2 cell related cytokines including IL-4, IL-5, and IL-13 are common in asthma related airway inflammation (Choy et al., 2015). Type 2 innate lymphoid cells (ILC2s), which are negative for lymphoid lineage markers, have recently been identified as an additional source of traditional Th2 cytokines (Licona-Limon et al., 2013; Doherty and Broide, 2015). ILC2s are regulated in part by IL-33 from epithelial cells, and have been suggested to be contributors to asthma (Hallstrand et al., 2013; Drake and Kita, 2014). Neutrophils, which can be recruited by IL-17 via CXCL1/KC induction, are also elevated in some subtypes of asthma (Choy et al., 2015; Lambrecht and Hammad, 2015). Allergens and other environmental exposures are common triggers for asthma once sensitization occurs (Kim and Mazza, 2011). Furthermore, low glutathione (GSH) levels have been shown to polarize the immune response to antigen exposure in the Th2 direction and reduce type 1 T-helper cell (Th1) cytokine production (Peterson et al., 1998; Kim et al., 2007).

Recent studies using mouse models of allergic airway disease (AAD) suggest that some engineered nanomaterials (ENMs) including silica, titanium and zinc oxide nanoparticles, and carbon nanotubes, can enhance sensitization to allergens. Most studies have used ovalbumin (OVA) and have either tested the adjuvant potential of ENMs in place of a traditional adjuvant (such as alum), or tested the ability for ENMs to augment OVA induced immune responses in the presence of a traditional adjuvant (Nygaard et al., 2009; Brandenberger et al., 2013; Jonasson et al., 2013; Roy et al., 2013). A few studies have used house dust mite extract (HDM), which is a clinically-relevant human allergen (Gregory and Lloyd, 2011; Piyadasa et al., 2016) that does not require an additional adjuvant. Studies have either examined the impact of other ENMs on existing AAD or examined whether co-exposure enhances sensitization (Cates et al., 2004; Ronzani et al., 2014; Shipkowski et al., 2015). However, the potential of QDs to impact allergic sensitization in mice has not been evaluated. In addition, genetic background, which can modulate susceptibility to QD-induced acute lung inflammation, has yet to be explored in the context of ENM enhancement of allergic sensitization (Scoville et al., 2015).

In this study, we tested the ability of QDs to enhance allergic sensitization to HDM in C57BL/6J and A/J mice. We also sought to explore the effect of HDM and QDs on levels of lung GSH and ILC2s. To our knowledge, this is the first study to enumerate lung ILC2s in A/J mice and compare them with another mouse strain in the context of airway responsiveness towards allergen and nanoparticle challenges. C57BL/6J and A/J mice were chosen because 1) they are genetically distinct, 2) C57BL/6J mice have been shown to be less susceptible than A/J mice to QD-induced acute lung inflammation (Scoville et al., 2015), and 3) C57BL/6J mice have naturally higher levels of lung GSH than A/J mice.

2. Methods

2.1. Mice and sensitization

All procedures were approved by the University of Washington Institutional Animal Care and Use Committee on protocol 2384-08. Seven-week old male C57BL/6J and A/J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed either 2 or 3 mice per cage in a specific pathogen free vivarium at the University of Washington and acclimated for a week prior to sensitization. Mice were on a 12 h light/dark cycle and food and water were available *ad libitum*. Groups of 6 mice from each strain were anesthetized with Isoflurane (4%, 2 L/min O₂ flow rate) and either sensitized on Day 0 to

50 μ L of saline with 2 μ g/ μ L HDM (Greer Laboratories, Lenoir NC – Cat.# RMB84M, lot# 275533) in saline (a total dose of 100 μ g HDM), or co-exposed to HDM + QDs (100 μ g HDM + 140 ng Cd equivalents of QDs) using oropharyngeal aspiration (OPA). On Days 8, 9, and 10, mice dosed on day 1 with saline were challenged with 50 μ L saline, while HDM and HDM + QD exposed mice received 50 μ L of 0.5 μ g/ μ L (a total dose of 25 μ g HDM) via OPA. One C57BL/6J HDM treated mouse was euthanized 8 days after OPA due to behavioral signs of distress. However, given that no other mice from any of the treatment groups showed any signs of distress after multiple OPA exposures, this was not considered HDM related. The dose of QDs in this study is virtually the same as the C57BL/6J and A/J mice received in our previous studies (Scoville et al., 2015).

2.2. Quantum dots

QDs used in this study were synthesized and characterized as previously described (Pellegrino et al., 2004; Bagalkot and Gao, 2011; McConnachie et al., 2012; McConnachie et al., 2013). The QDs are composed of a cadmium/selenium core and a zinc sulfide shell. The QDs, stabilized with a layer of surface ligand (tri-n-octylphosphine oxide), are solubilized with an amphiphilic polymer, poly(maleic anhydride-*alt*-1-tetradecene) (TOPO-PMAT).

2.3. Lung mechanics

On Day 11, mice were anesthetized with pentobarbital (90 mg/kg body weight) and intubated via tracheotomy with an 18 gauge Luer stub secured in the trachea with a suture. The mice were then ventilated using a flexiVent apparatus (SCIREQ Inc., Montreal, QC, Canada) to assess lung function. After an injection of pancuronium (0.1 mL) to eliminate breathing reflexes, pulmonary function was evaluated. The lungs were ventilated with a tidal volume of 10 mL/kg, at a rate of 180 breaths/min, with a positive end expiratory pressure of 3 cm H₂O. After a total lung capacity maneuver was performed at 30 cm H₂O, baseline measurements were collected. In order to assess airway hyperresponsiveness (AHR), progressive doses of nebulized methacholine (0, 3.125, 12.5, and 50 mg/mL) were delivered to the lungs. At each dose of methacholine (MTCH), a 3 s multi-frequency forced oscillation maneuver was applied for determination of total lung impedance followed by a 1.5 s single frequency oscillation for determination of Resistance (R), Elastance (E), and Compliance (C). Airway Resistance (Rn), Tissue Damping (G), and Tissue Elastance (H) were calculated by the flexiVent software by applying the Constant Phase model to the impedance measurement. Area under the curve was calculated for each animal to allow for strain and treatment comparisons (Vanoirbeek et al., 2010).

2.4. Bronchoalveolar lavage (BAL)

Three lavages were performed in each mouse using 1 mL phosphate buffered saline (PBS). Cells were collected by centrifugation of the recovered BAL fluid (BALF) at 300g for 5 min at 4 °C. The supernatant from the 1st lavage in each mouse was stored at –80 °C for measuring total BALF protein and cytokines. Cells from all three lavages were combined in 500 μ L RPMI media with 10% fetal bovine serum (FBS) and counted using a Cellometer Auto 2000 (Nexcelom Bioscience, Lawrence, MA). Aliquots of BAL cells (20,000 cells/slide) were centrifuged at 750 RPM for 5 min using a Cytopro model 7620 cytocentrifuge (Wescor Inc., Logan, UT) and later stained with Diff-Quik (Siemens - Thermo Fisher Scientific, Waltham, MA).

2.5. Lung tissue collection and processing

Following lavage, the lungs were perfused with Hank's balanced salt solution (HBSS). The left lung was inflated and fixed with 10% neutral buffered formalin. The right superior and inferior lobes were collected

and placed on ice in Roswell Park Memorial Institute (RPMI) medium with 10% FBS. After removal of medium, lung tissue was minced with scissors. A small portion was frozen for future protein analyses and another was placed into Trizol (Invitrogen, Carlsbad, CA) and frozen for future mRNA extraction. Digestion medium (RPMI, 5% FBS, 0.5 mg/mL Liberase (Roche, Indianapolis, IN), 40 U/mL DNase 1 (Qiagen, Germantown, MD)) was added to the remaining minced lung tissue. Following a 30 min incubation at 37 °C, the digested lung mixture was filtered through a 40 µm filter (Corning-Falcon, Corning Inc., Corning, NY) to obtain a single-cell suspension. The accessory and middle right lobes were flash frozen for measurement of GSH.

2.6. Multi-color flow cytometry for BALF cell differentials

BALF single cell suspensions were stained for enumeration of inflammatory cell populations as previously described (Nolin et al., 2016). Briefly, BALF cells were blocked using Fc block (BD Biosciences, San Jose, CA) and then labeled with an anti-mouse antibody cocktail (CD45-APC/Cy7¹, CD3-PerCP-Cy5.5², MHC-II -BV605³, SiglecF-PE³, Cd11c-FITC², and Gr1-APC² – ¹Biolegend, San Diego, CA, ²eBiosciences, San Diego, CA, ³BD Biosciences, San Jose, CA). Cells were also incubated with eFluor 450 fixable viability dye (eBioscience). Single stained controls used for color compensation were obtained using ArC amine reactive beads (Thermo-Fisher, Waltham, MA) and viability of single stained controls was obtained using OneComp eBeads (eBioscience). Stained cells were analyzed using a FACS LSR II flow cytometer (Becton Dickinson Instrument Systems, Franklin Lakes, NJ). Gating and color compensation were performed using FlowJo software (TreeStar, Ashland, OR).

2.7. Multi-color flow cytometry for lung ILC2s

For detection of ILC2s, lung single cell suspensions were labeled with an anti-mouse antibody cocktail (Mouse Hematopoietic Lineage FITC Cocktail (CD3, CD45R/B220, CD11b, Ter119, Gr-1), CD45 (AlexaFluor 700² or APC/Cy7¹), CD90.2 (APC²), CD25(BV421¹), and ST2 (PE³) – ¹Biolegend, San Diego, CA; ²eBiosciences, San Diego, CA; ³MD Biosciences, St Paul, MN). Cells were treated with Fc Block (BD Bioscience) and subsequently incubated with the primary antibody cocktail for 30 min at 4 °C in the dark. After washing, cells were re-suspended in PBS containing eFluor 450UV fixable viability dye (eBioscience) and incubated for 15 min at 4 °C in the dark. Cells were fixed in 10% neutral buffered formalin and stored at 4 °C in MACS buffer until analyzed via flow cytometry. ArC amine reactive beads and OneComp eBeads were used for single stain compensation controls as described above. Fluorescence minus one (FMO) controls for CD90.2, CD25, and ST2 were also used to set the gating thresholds. All FMOs contained the lineage cocktail and the CD45 antibody with the omission of only CD90.2, CD25 or ST2 to allow for accurate gating. All samples were analyzed using a BD FACS LSR II flow cytometer and subsequent gating and compensation were performed using FlowJo.

2.8. BALF total protein and cytokine analysis

Total protein levels in BALF were measured in triplicate using the Bio-Rad Protein Assay (BioRad, Hercules, CA) according to manufacturer's instructions. Absorbance was read at 590 nm using a SpectraMax 190 spectrophotometric plate reader (Molecular Devices, Sunnyvale, CA). Protein concentrations were interpolated from a standard curve created using dilutions of a bovine serum albumin solution (0–0.35 mg/mL). BALF cytokines interferon gamma (IFN-γ), interleukin 5 (IL-5), interleukin 17 A (IL-17A), interleukin 33 (IL-33), keratinocyte chemoattractant (KC), and interleukin 10 (IL-10) were measured in duplicate using an electrochemiluminescence based U-Plex assay according to the manufacturer's recommended protocol (Meso Scale Discovery - Meso Scale Diagnostics, Rockville, MD).

Chemiluminescence signals were measured with a Sector Image 2400 microplate detection system (Meso Scale Discovery). Samples below the lower limit of detection (LLOD) were replaced with the analyte-specific LLOD divided by the square root of 2.

2.9. Lung total glutathione

Approximately 20–40 mg of lung tissue was homogenized in 400 µL of TES/SB buffer (20 mM Tris, pH 7.4, 1 mM EDTA, 250 mM sucrose, 20 mM serine, and 1 mM boric acid with 10 µL/mL protease inhibitor (Roche, Indianapolis, IN)) using 5 mm steel beads (Qiagen, Germantown, MD) in a 2 mL micro-tube for 5 min at 50 Hz using a TissueLyser (Qiagen). Levels of glutathione were measured as previously described (Weldy et al., 2011). Briefly, glutathione in lung tissue samples and standards (0–0.25 mM) was reduced with tris(2-carboxyethyl) phosphine, and then derivatized with naphthene-2,3-dicarboxaldehyde. Relative fluorescence intensity was measured for samples and standards. Sample concentrations were interpolated from the GSH standard curve. Standards and samples were analyzed in triplicate and samples were normalized to total protein levels in the lung tissue sample measured using the Bradford method.

2.10. Statistical analysis

Differences between strains and treatment groups were analyzed using two-way ANOVA on log transformed data. If a significant QD treatment effect or interaction was seen using two-way ANOVA then a one-way ANOVA was performed in each strain and post-hoc comparisons were made using Tukey's HSD test. Pearson's correlation coefficients between endpoints were calculated using r.corr function in the Hmisc R package (Harrell et al., 2016). Data were managed in Microsoft Excel and R. All analyses were done using R and plots were generated using ggplot2 (Wickham, 2009) and corplot (Wei and Simko, 2016).

3. Results

3.1. AHR and lung permeability

Similar to Nolin et al. (Nolin et al., 2016), AHR was assessed measuring total lung resistance (R) during MTCH challenge (Fig. 1A). We did not observe a significant difference in AHR, defined by total lung resistance, in either strain. However, A/J mice were observed to be significantly more responsive to MTCH than C57BL/6J mice over all. Interestingly, while it appears that HDM and HDM + QD exposed C57BL/6J mice may have increased R compared to the saline group, the differences were not significant. Lung permeability was assessed by measuring total protein levels in BALF (Fig. 1B). Significant increases in total BALF protein were observed in HDM + QD co-exposed C57BL/6J and A/J mice but not in mice exposed to HDM alone. In C57BL/6J mice, a weak but significant association between levels of BALF protein and R was observed (Fig. 1C). Additional lung mechanics measurements are shown in Fig. 1S.

3.2. BALF inflammatory cells

To characterize lung inflammatory cells, multiple CD45⁺ BALF cell populations were enumerated using flow cytometry (Fig. 2). Total CD45⁺ cells, T-cells, eosinophils and neutrophils, were all significantly increased in HDM and HDM + QD groups relative to saline in both C57BL/6J and A/J mice (Fig. 2 A, E, F–H). For dendritic cells, a marginal QD treatment effect ($p = .06$) and interaction ($p = .06$) were observed (Fig. 2C). Both C57BL/6J and A/J mice showed histopathological changes consistent with an allergic response to HDM exposure (Fig. 2S).

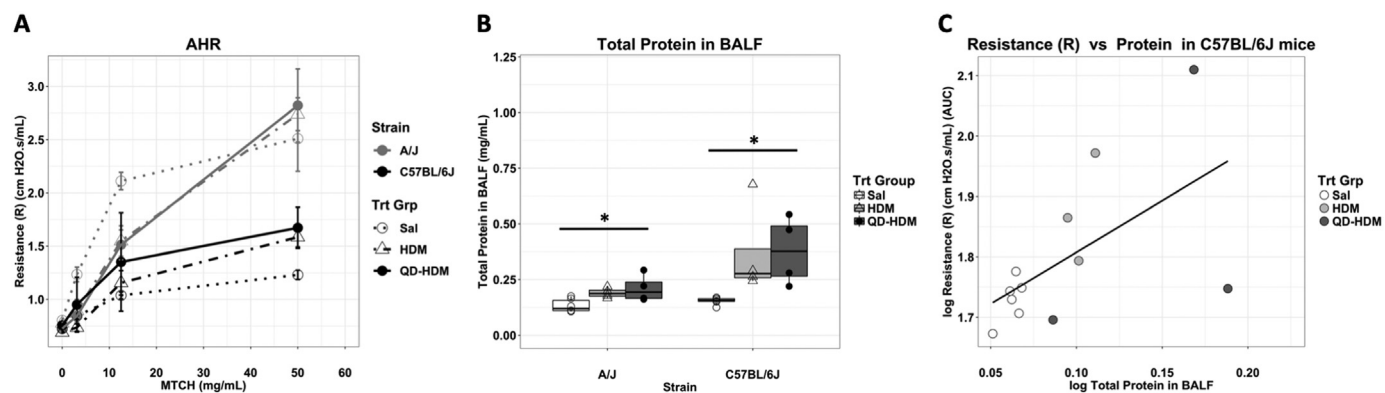


Fig. 1. Levels of total lung resistance (R) in different strain and treatment groups are shown as means $\pm$ SEM (A). Concentrations of total BALF protein for each mouse are shown overlaid on a box plot (B). Correlation of R and total BALF protein in C57BL/6J mice (C). The linear regression line is plotted along with the r^2 and p -value of the association (C). * $q < 0.05$ from Tukey's HSD test.

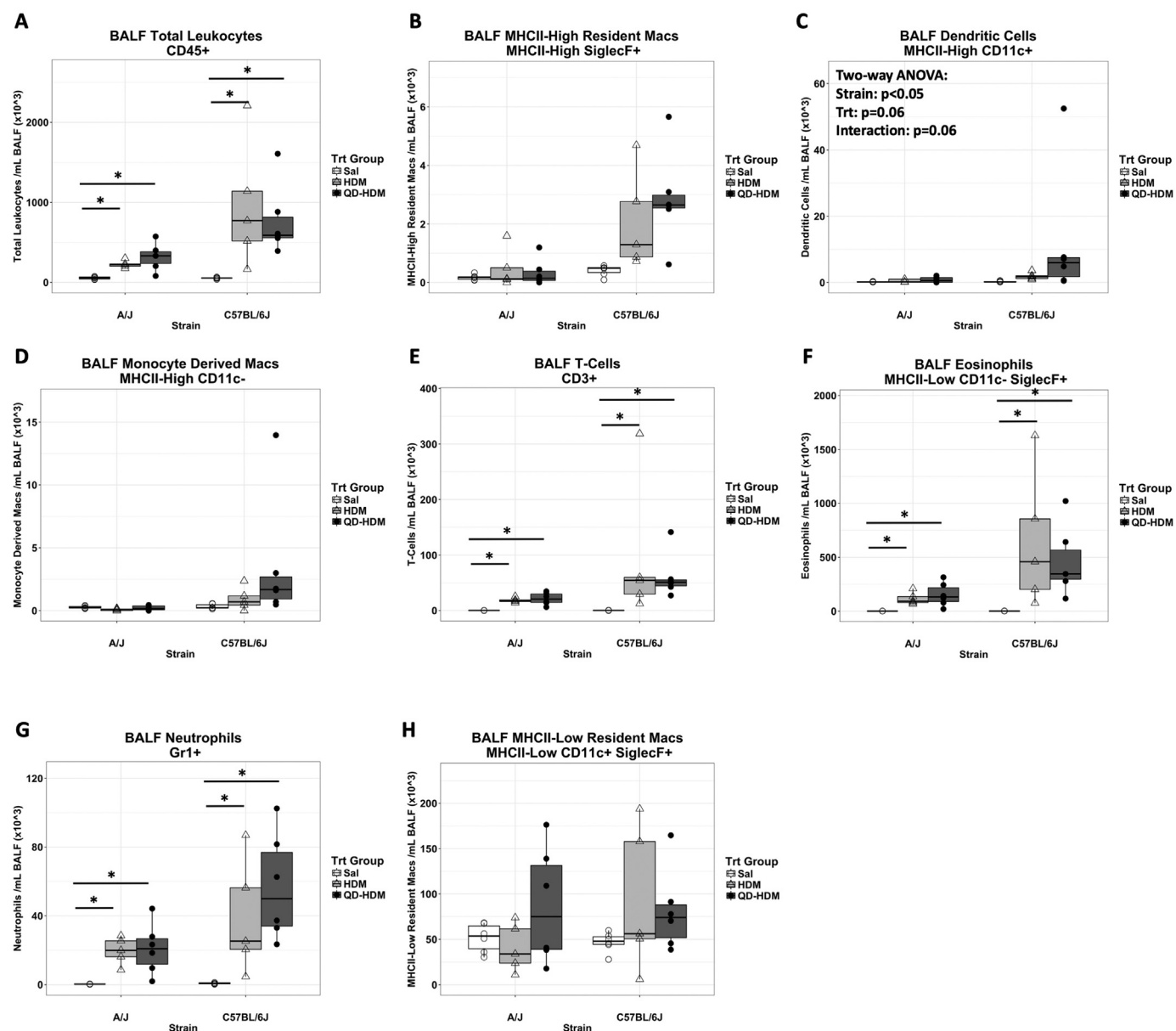


Fig. 2. Concentrations of total CD45⁺ leukocytes (A), MHC-II^{high} resident macrophages (B), dendritic cells (C), monocyte derived macrophages (D), T-cells (E), eosinophils (F), neutrophils (G), and MHC-II^{low} resident macrophages (H) for each mouse are shown overlaid on a box plot. * $q < 0.05$ from Tukey's HSD test.

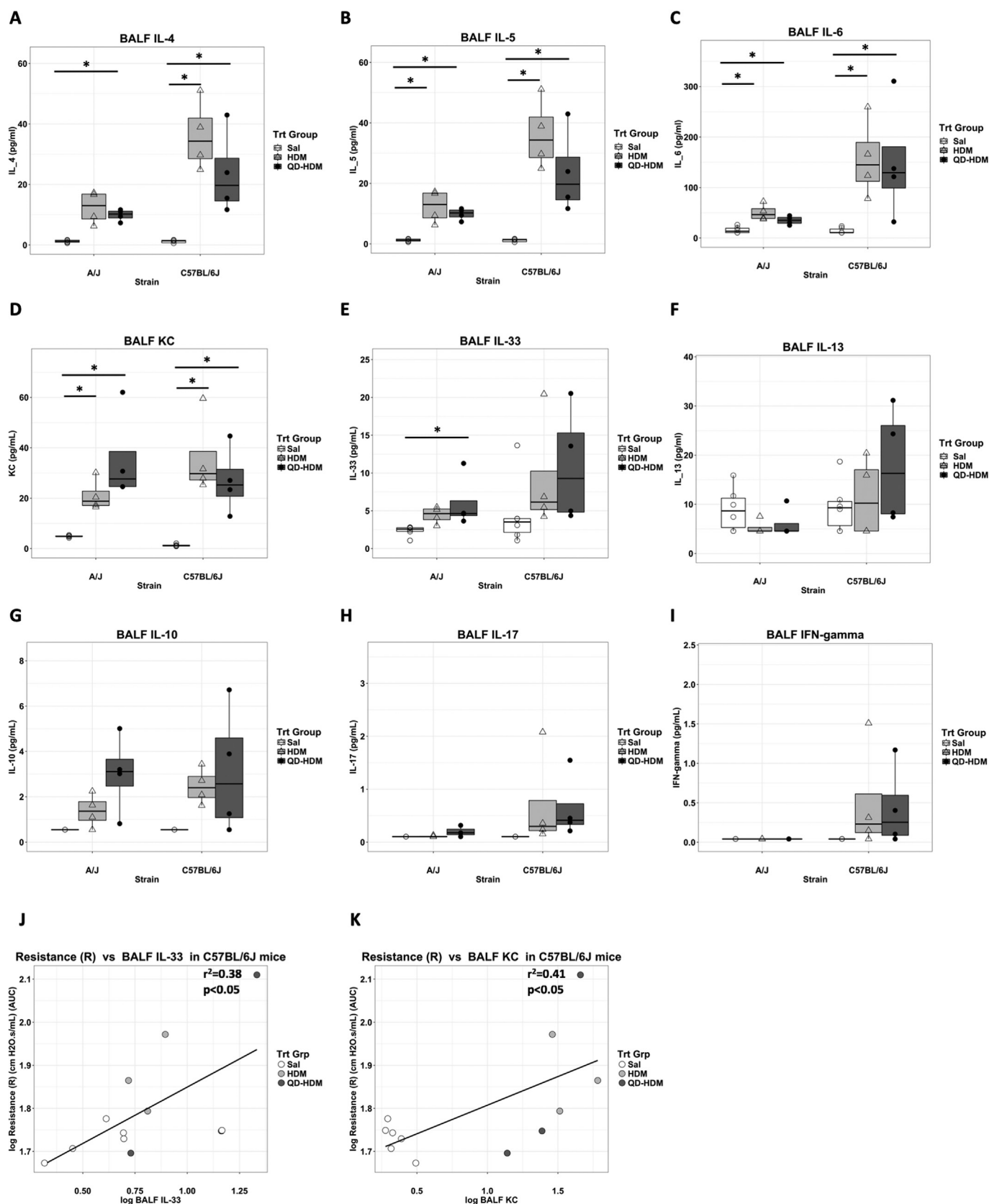


Fig. 3. Concentrations of BALF IL-4 (A), IL-5 (B), IL-6 (C), KC (D), IL-33 (E), IL-13 (F), IL-10 (G), IL-17 (H), and IFN- γ (I) for each mouse are shown overlaid on a box plot (A-I). Correlations of R and IL-33 (J) and KC (K). The linear regression line is plotted along with the r^2 and p-value of the association. * $q < 0.05$ from Tukey's HSD test.

3.3. BALF cytokines

To further characterize HDM and QD + HDM-induced inflammatory responses, BALF cytokines IFN- γ , IL-4, IL-5, IL-6, IL-10, IL-17, IL-13, IL-33, and KC were measured using MSD U-plex assays (Fig. 3). IL-4, IL-5, IL-6, and KC were significantly induced by both HDM and HDM + QD co-exposure compared to saline in both C57BL/6J and A/J mice (Fig. 3 A-D). IL-33 was significantly increased by HDM + QD co-exposure but not by HDM alone compared to saline in A/J mice (Fig. 3E). Levels of IL-13 were detectable in all QD + HDM co-exposed C57BL/6J mice, some HDM and saline treated C57BL/6J and in some A/J mice (Fig. 3F). Levels of IL-10 were detectable in HDM treated and HDM + QD co-exposed A/J and C57BL/6J mice but undetectable in saline treated mice from either strain (Fig. 3G). IL-17 was detectable in all HDM treated and HDM + QD co-exposed C57BL/6J mice, some HDM treated and HDM + QD co-exposed A/J mice, and undetectable in all saline treated mice (Fig. 3H). IFN- γ was only detectable in HDM sensitized and QD + HDM co-exposed C57BL/6J mice (Fig. 3I). Significant positive associations were observed between R and IL-33 and KC in C57BL/6J mice (Fig. 3J and Fig. 3K).

3.4. Lung ILC2 cells and glutathione

ILC2 cells were identified by gating for CD45⁺, CD90.2⁺ CD25⁺ and ST2⁺ (IL-33 receptor). While neither HDM nor HDM + QD co-exposure changed the numbers of ILC2 cells, A/J mice were observed to

have significantly more lung ILC2 cells than C57BL/6J mice overall (Fig. 4A). GSH is essential for redox homeostasis and redox status has been associated with immune response polarization. No significant differences in lung tissue GSH among saline, HDM sensitized or HDM + QD co-exposed mice in either strain were found (Fig. 4B). However, significant inverse associations between levels of GSH and lung ILC2 cells and MHC-II^{low} resident macrophages were observed in A/J mice (Fig. 4C, Fig. 4D).

4. Discussion

While recent studies have shown that some ENMs can enhance allergic sensitization in mouse models of AAD, the potential for QDs to act in a similar manner has not been evaluated. Genetic background as a susceptibility factor to ENM enhancement of allergic sensitization has also not yet been explored. Thus, in this study we sensitized C57BL/6J and A/J mice to the murine and human allergen HDM and HDM + QDs to test the hypotheses that QDs would enhance allergic sensitization to HDM and that genetic background would modulate susceptibility.

In this short-term sensitization and challenge model, we did not see robust AHR, but did see significant increases in BALF eosinophils IL-4, and IL-5 in BALF in HDM sensitized mice of both strains, indicative of type-2 airway inflammation. Levels of IL-5, eosinophils and neutrophils in BALF in mice from our study were comparable to levels in another recent study investigating HDM sensitization in BALB/c mice (Piyadasa et al., 2016). While not significant, there is a suggestion that levels of

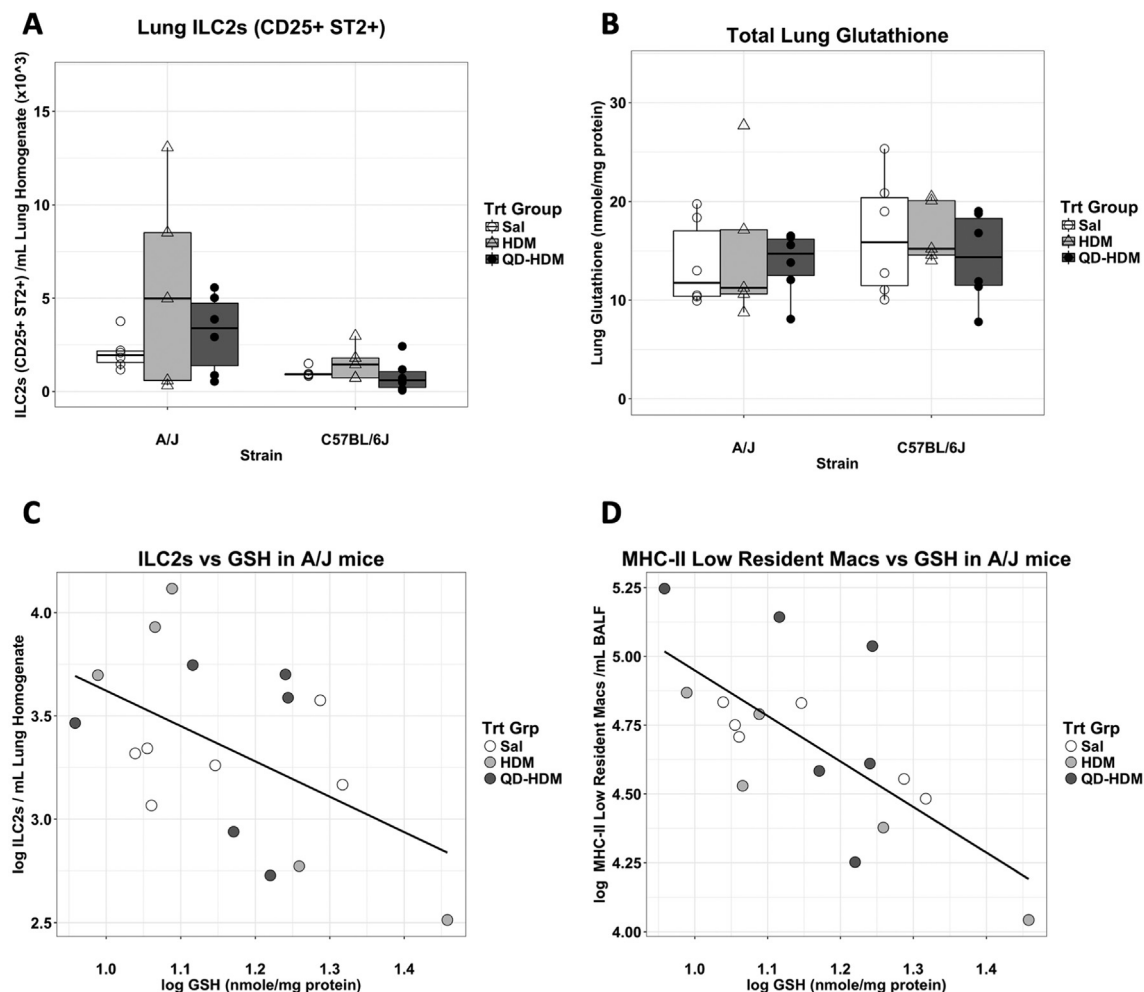


Fig. 4. Levels of lung ILC2s and glutathione for each mouse are shown overlaid on a box plot (A and B). Correlations between ILC2s (C), and MHC-II^{low} resident macrophages (D), and lung GSH in A/J mice. The linear regression line is plotted along with the r^2 and p-value of the association. * $q < 0.05$ from Tukey's HSD test.

total lung resistance R may be increased by HDM sensitization in C57BL/6J mice, and levels of total protein, IL-33 and KC in C57BL/6J mice were significantly correlated with R. This may suggest that in addition to IL-33, known to contribute to AHR, increased lung permeability and KC are also contributing to R in C57BL/6J mice (Barlow et al., 2013; Sjöberg et al., 2017).

Interestingly, we found that levels of total protein in BALF were significantly elevated in mice from both mouse strains co-exposed to HDM + QD during sensitization but not in mice sensitized to HDM alone, suggesting that QDs may have enhanced the effects of HDM on lung permeability. Furthermore, in A/J mice, BALF IL-33 was significantly increased by HDM + QD co-exposure during sensitization but not with HDM alone, suggesting that QDs may also enhance HDM-induced select cytokine responses in a strain dependent manner.

In addition to the hallmark cytokines IL-4 and IL-5, IL-6 has also been shown to contribute to Th2 responses in the lung and was increased in HDM treated and HDM + QD co-exposed mice in both strains (Diehl and Rincón, 2002; Rincon and Irvin, 2012). IFN- γ and IL-17 were essentially only detectable in HDM and HDM + QD groups of C57BL/6J mice, suggesting that C57BL/6J may also be mounting Th1 and Th17 responses. IL-10 was detected in HDM treated and HDM + QD co-exposed A/J and C57BL/6J mice but not in saline groups, which suggest that both strains may be simultaneously trying to limit their inflammatory responses (Saraiva and O'Garra, 2010). IL-13, which is an additional Th2 cytokine, was detectable in all QD + HDM co-exposed C57BL/6J mice and there was a suggestive but not significant trend towards an increase in HDM and HDM + QD co-exposed compared to saline. Surprisingly, this was pattern was not present in A/J mice. Furthermore, several cytokines and BAL cell populations that were significantly increased in HDM and HDM + QD groups in both strains, appear to be lower in A/J mice compared to correspondingly treated C57BL/6J mice. While there are multiple known sources for most cytokines, it has recently been shown that depleting blood monocytes, which can differentiate into macrophages in tissues, significantly lowers the levels of HDM-induced inflammatory cells and cytokines (Zasłona et al., 2014; Jakubczik et al., 2017). In this study, C57BL/6J mice showed a trend, although not statistically significant, towards increased levels of monocyte-derived macrophages in HDM treated and HDM + QD co-exposed compared to saline treated mice. In contrast, there were no apparent changes in monocyte derived macrophages in A/J mice across treatment groups, which could explain at least in part why the A/J mice appeared to show reduced cytokine and cellular responses to HDM and HDM + QD co-exposure compared to C57BL/6J mice.

We also found that A/J mice have significantly higher numbers of lung ILC2 cells overall than C57BL/6J mice. While we did not see significant differences in the numbers of ILC2s among treatment groups for either strain, to our knowledge this is the first study to enumerate ILC2s in A/J mice and compare their numbers to a different mouse strain (C57BL/6J) that is commonly used in allergic lung inflammation studies. In addition, a significant inverse correlation was observed between lung GSH levels and ILC2s in A/J mice despite a lack of significant changes in GSH levels across treatment groups. GSH levels were also inversely correlated in A/J mice with MHC-II^{low} resident macrophages. Both correlations are intriguing given that glutathione levels are known to influence Th2 immune responses, although the exact nature of the relationship is still not entirely clear (Peterson et al., 1998; Chan et al., 2006; Kim et al., 2007; Lowry et al., 2008).

Limitations of this study mainly arise from the short-term sensitization design, which was conceived as a pilot study into potential QD enhancement of HDM allergic sensitization; the potential impact on glutathione levels and ILC2s; and whether such effects would be dependent on genetic background. Accordingly, it is possible that this time frame was too brief and the number of HDM and HDM + QD exposures too few for AHR to fully develop. In keeping with the pilot nature of this study, we also did not directly assess QD adjuvant activity

immunologically (e.g. antigen specific T-cell populations and/or IgE). Despite these limitations, results from this study suggest that 1) QDs influence HDM induced AAD by potentiating and/or enhancing select cytokine production; 2) that genetic background modulates the impact of QDs on HDM sensitization; and 3) that potential ILC2 contributions to HDM induced AAD are also likely modulated by genetic background. These findings raise concerns for humans occupationally exposed to QDs, especially among those developing allergic airway disease and warrant future studies into QD enhancement of HDM sensitization with longer-term exposure paradigms where definitive measures of immunological adjuvant activity and the importance of recruited monocytes should be assessed in an expanded panel of mouse strains.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.taap.2019.01.018>.

References

- Bagalkot, V., Gao, X., 2011. siRNA-aptamer Chimeras on Nanoparticles: Preserving Targeting Functionality for Effective Gene Silencing. *ACS Nano* 5 (10), 8131–8139.
- Barlow, J.L., Peel, S., Fox, J., Panova, V., Hardman, C.S., Camelo, A., McKenzie, A.N., 2013. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J. Allergy Clin. Immunol.* 132 (4), 933–941.
- Brandenberger, C., Rowley, N.L., Jackson-Humbles, D.N., Zhang, Q., Bramble, L.A., Lewandowski, R.P., Harkema, J.R., 2013. Engineered silica nanoparticles act as adjuvants to enhance allergic airway disease in mice. *Particle and Fibre Toxicology* 10 (1), 26.
- Cartwright, M.M., Schmuck, S.C., Corredor, C., Wang, B., Scoville, D.K., Chisholm, C.R., Kavanagh, T.J., 2016. The pulmonary inflammatory response to multiwalled carbon nanotubes is influenced by gender and glutathione synthesis. *Redox Biol.* 9, 264–275.
- Cates, E.C., Fattouh, R., Wattie, J., Inman, M.D., Goncharova, S., Coyle, A.J., Jordana, M., 2004. Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J. Immunol.* 173 (10), 6384–6392.
- CDC, 2017. CDC - Asthma - Data and Surveillance - Asthma Surveillance Data. from. <http://www.cdc.gov/asthma/asthmaadata.htm>.
- Chan, R.C.-F., Wang, M., Li, N., Yanagawa, Y., Onoé, K., Lee, J.J., Nel, A.E., 2006. Pro-oxidative diesel exhaust particle chemicals inhibit LPS-induced dendritic cell responses involved in T-helper differentiation. *J. Allergy Clin. Immunol.* 118 (2), 455–465.
- Choy, D.F., Hart, K.M., Borthwick, L.A., Shikotra, A., Nagarkar, D.R., Siddiqui, S., Bradding, P., 2015. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. *Sci. Transl. Med.* 7 (301), 301ra129.
- Cui, L., 2013. Exposure Assessment and Inflammatory Response among Workers Producing Calcium Carbonate Nanomaterials Dissertation. University of Washington.
- Diehl, S., Rincón, M., 2002. The two faces of IL-6 on Th1/Th2 differentiation. *Mol. Immunol.* 39 (9), 531–536.
- Doherty, T.A., Broide, D.H., 2015. Group 2 innate lymphoid cells: new players in human allergic diseases. *J. Invest. Allergol. Clin. Immunol.* 25 (1), 1–11.
- Drake, L.Y., Kita, H., 2014. Group 2 innate lymphoid cells in the lung. *Adv. Immunol.* 124, 1–16.
- Gregory, L.G., Lloyd, C.M., 2011. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol.* 32 (9), 402–411.
- Hallstrand, T.S., Hackett, T.L., Altmeier, W.A., Matute-Bello, G., Hansbro, P.M., Knight, D.A., 2013. Airway epithelial regulation of pulmonary immune homeostasis and inflammation. *Clin. Immunol.* 151 (1), 1–15.
- Harrell, F.J.J., Dupont, C., et al., 2016. Hmisc. from. <https://CRAN.R-project.org/package=Hmisc>.
- Hu, X., Han, H., Hua, L., Sheng, Z., 2010. Electrogenated chemiluminescence of blue emitting ZnSe quantum dots and its biosensing for hydrogen peroxide. *Biosens. Bioelectron.* 25 (7), 1843–1846.
- Jakubczik, C.V., Randolph, G.J., Henson, P.M., 2017. Monocyte differentiation and antigen-presenting functions. *Nat. Rev. Immunol.* 17, 349.
- Jonasson, S., Gustafsson A Fau - Koch, B., Koch B Fau - Bucht, A., Bucht, A., 2013. Inhalation exposure of nano-scaled titanium dioxide (TiO₂) particles alters the inflammatory responses in asthmatic mice. *Inhal. Toxicol.* 25 (4), 1091–1099.

- Jung, H., Chung, W., Lee, C.H., Kim, S.H., 2012. Fabrication of white light-emitting diodes based on UV light-emitting diodes with conjugated polymers-(CdSe/ZnS) quantum dots as hybrid phosphors. *J. Nanosci. Nanotechnol.* 12 (7), 5407–5411.
- Kim, H., Mazza, J., 2011. Asthma. *Allergy, Asthma Clin. Immunol.* 7 (1), S2.
- Kim, S., Lim, Y.T., Soltesz, E.G., De Grand, A.M., Lee, J., Nakayama, A., Frangioni, J.V., 2004. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat. Biotechnol.* 22 (1), 93–97.
- Kim, H.-J., Barajas, B., Chan, R.C.-F., Nel, A.E., 2007. Glutathione depletion inhibits dendritic cell maturation and delayed-type hypersensitivity: Implications for systemic disease and immunosenescence. *J. Allergy Clin. Immunol.* 119 (5), 1225–1233.
- Lambrecht, B.N., Hammad, H., 2015. The immunology of asthma. *Nat. Immunol.* 16 (1), 45–56.
- Liao, H.-Y., Chung, Y.-T., Lai, C.-H., Wang, S.-L., Chiang, H.-C., Li, L.-A., Liou, S.-H., 2014. Six-month follow-up study of health markers of nanomaterials among workers handling engineered nanomaterials. *Nanotoxicology* 8 (sup1), 100–110.
- Licona-Limon, P., Kim, L.K., Palm, N.W., Flavell, R.A., 2013. TH2, allergy and group 2 innate lymphoid cells. *Nat. Immunol.* 14 (6), 536–542.
- Lowry, M.H., McAllister, B.P., Jean, J.-C., Brown, L.A.S., Hughey, R.P., Cruikshank, W.W., Joyce-Brady, M., 2008. Lung lining fluid glutathione attenuates IL-13-induced asthma. *Am. J. Respir. Cell Mol. Biol.* 38 (5), 509–516.
- Ma-Hock, L., Treumann, S., Strauss, V., Brill, S., Luiz, F., Mertler, M., Landsiedel, R., 2009. Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. *Toxicol. Sci.* 112 (2), 468–481.
- Ma-Hock, L., Brill, S., Wohlleben, W., Farias, P.M.A., Chaves, C.R., Tenório, D.P.L.A., van Ravenzwaay, B., 2012. Short term inhalation toxicity of a liquid aerosol of CdS/Cd(OH)2 core shell quantum dots in male Wistar rats. *Toxicol. Lett.* 208 (2), 115–124.
- McConnachie, L.A., White, C.C., Botta, D., Zadworny, M.E., Cox, D.P., Beyer, R.P., Kavanagh, T.J., 2012. Heme oxygenase expression as a biomarker of exposure to amphiphilic polymer-coated CdSe/ZnS quantum dots. *Nanotoxicology* 7 (2), 181–191.
- McConnachie, L.A., Botta, D., White, C.C., Weldy, C.S., Wilkerson, H.W., Yu, J., Kavanagh, T.J., 2013. The glutathione synthesis gene gclm modulates amphiphilic polymer-coated CdSe/ZnS quantum dot-induced lung inflammation in mice. *PLoS One* 8 (5), e64165.
- Medintz, I.L., Uyeda, H.T., Goldman, E.R., Mattoussi, H., 2005. Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater.* 4, 435–446.
- Nolin, J.D., Ogden, H.L., Lai, Y., Altemeier, W.A., Frevert, C.W., Bollinger, J.G., Hallstrand, T.S., 2016. Identification of epithelial phospholipase A2 receptor 1 (PLA2R1) as a potential target in asthma. *Am. J. Respir. Cell Mol. Biol.* 6, 825–826.
- Nygaard, U.C., Hansen, J.S., Samuelsen, M., Alber, T., Marioara, C.D., Løvik, M., 2009. Single-walled and multi-walled carbon nanotubes promote allergic immune responses in mice. *Toxicol. Sci.* 109 (1), 113–123.
- Pellegrino, T., Manna, L., Kudera, S., Liedl, T., Koktysh, D., Rogach, A.L., Parak, W.J., 2004. Hydrophobic nanocrystals coated with an amphiphilic polymer shell: a general route to water soluble nanocrystals. *Nano Lett.* 4 (4), 703–707.
- Peterson, J.D., Herzenberg, L.A., Vasquez, K., Waltenbaugh, C., 1998. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc. Natl. Acad. Sci. U. S. A.* 95 (6), 3071–3076.
- Piyadasa, H., Altieri, A., Basu, S., Schwartz, J., Halayko, A.J., Mookherjee, N., 2016. Biosignature for airway inflammation in a house dust mite-challenged murine model of allergic asthma. *Biology Open* 5 (2), 112–121.
- Rincon, M., Irvin, C.G., 2012. Role of IL-6 in asthma and other inflammatory pulmonary diseases. *Int. J. Biol. Sci.* 8 (9), 1281–1290.
- Roberts, J.R., Antonini, J.M., Porter, D.W., Chapman, R.S., Scabilloni, J.F., Young, S.H., Mercer, R.R., 2013. Lung toxicity and biodistribution of Cd/Se-ZnS quantum dots with different surface functional groups after pulmonary exposure in rats. *Particle and Fibre Toxicology* 10, 5.
- Ronzani, C., Casset, A., Pons, F., 2014. Exposure to multi-walled carbon nanotubes results in aggravation of airway inflammation and remodeling and in increased production of epithelium-derived innate cytokines in a mouse model of asthma. *Arch. Toxicol.* 88 (2), 489–499.
- Roy, R., Kumar, S., Verma, A.K., Sharma, A., Chaudhari, B.P., Tripathi, A., Dwivedi, P.D., 2013. Zinc oxide nanoparticles provide an adjuvant effect to ovalbumin via a Th2 response in Balb/c mice. *Int. Immunol.* 26 (3), 159–172.
- Saraiva, M., O'Garra, A., 2010. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10 (3), 170–181.
- Scoville, D.K., White, C.C., Botta, D., McConnachie, L.A., Zadworny, M.E., Schmuck, S.C., Kavanagh, T.J., 2015. Susceptibility to quantum dot induced lung inflammation differs widely among the Collaborative Cross founder mouse strains. *Toxicol. Appl. Pharmacol.* 289 (2), 240–250.
- Seiffert, J., Hussain, F., Wiegman, C., Li, F., Bey, L., Baker, W., Chung, K.F., 2015. Pulmonary toxicity of instilled silver nanoparticles: influence of size, coating and rat strain. *PLoS One* 10 (3), e0119726.
- Shipkowski, K.A., Taylor, A.J., Thompson, E.A., Glista-Baker, E.E., Sayers, B.C., Messenger, Z.J., Bonner, J.C., 2015. An allergic lung microenvironment suppresses carbon nanotube-induced inflammasome activation via STAT6-dependent inhibition of caspase-1. *PLoS One* 10 (6), e0128888.
- Silva, R.M., Anderson, D.S., Franz, L.M., Peake, J.L., Edwards, P.C., Van Winkle, L.S., Pinkerton, K.E., 2015. Pulmonary effects of silver nanoparticle size, coating, and dose over time upon intratracheal instillation. *Toxicol. Sci.* 144 (1), 151–162.
- Sjöberg, L.C., Nilsson, A.Z., Lei, Y., Gregory, J.A., Adner, M., Nilsson, G.P., 2017. Interleukin 33 exacerbates antigen driven airway hyperresponsiveness, inflammation and remodeling in a mouse model of asthma. *Sci. Rep.* 7 (1), 4219.
- Vanoirbeek, J.A., Rinaldi, M., De Vooght, V., Haenen, S., Bobic, S., Gayan-Ramirez, G., Janssens, W., 2010. Noninvasive and invasive pulmonary function in mouse models of obstructive and restrictive respiratory diseases. *Am. J. Respir. Cell Mol. Biol.* 42 (1), 96–104.
- Wang, X., Katwa, P., Podila, R., Chen, P., Ke, P.C., Rao, A.M., Brown, J.M., 2011. Multi-walled carbon nanotube instillation impairs pulmonary function in C57BL/6 mice. *Particle and Fibre Toxicology* 8 (1), 24.
- Wei, T., Simko, V., 2016. **Corrplot: Visualization of a Correlation Matrix** 2016. from. <https://github.com/taiyun/corrplot>.
- Weldy, C.S., White, C.C., Wilkerson, H.W., Larson, T.V., Stewart, J.A., Gill, S.E., Kavanagh, T.J., 2011. Heterozygosity in the glutathione synthesis gene Gclm increases sensitivity to diesel exhaust particulate induced lung inflammation in mice. *Inhal. Toxicol.* 23 (12), 724–735.
- Wickham, H., 2009. *ggplot2: Elegant Graphics for Data Analysis*. Springer Publishing Company (Incorporated).
- Wu, X., Liu, H., Liu, J., Haley, K.N., Treadway, J.A., Larson, J.P., Bruchez, M.P., 2003. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nat. Biotechnol.* 21 (1), 41–46.
- Zasłona, Z., Przybranowski, S., Wilke, C., van Rooijen, N., Teitz-Tennenbaum, S., Osterholzer, J.J., Peters-Golden, M., 2014. Resident alveolar macrophages suppress, whereas recruited monocytes promote, allergic lung inflammation in murine models of asthma. *J. Immunol.* 193 (8), 4245.

Supplemental Information

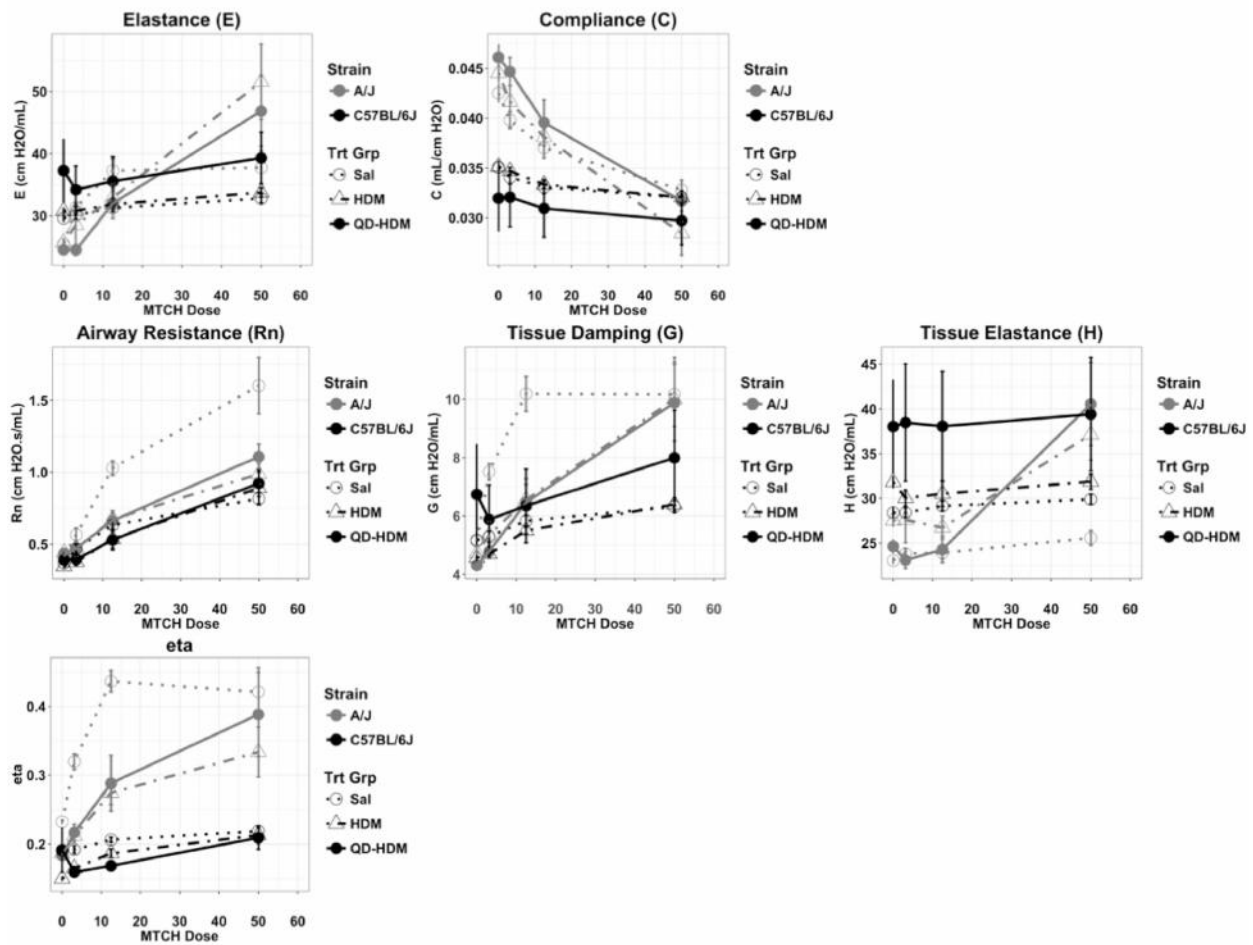


Figure S1. Additional measures of lung mechanics in saline, HDM-sensitized, and HDM+QD co-exposed C57BL/6J and A/J mice.

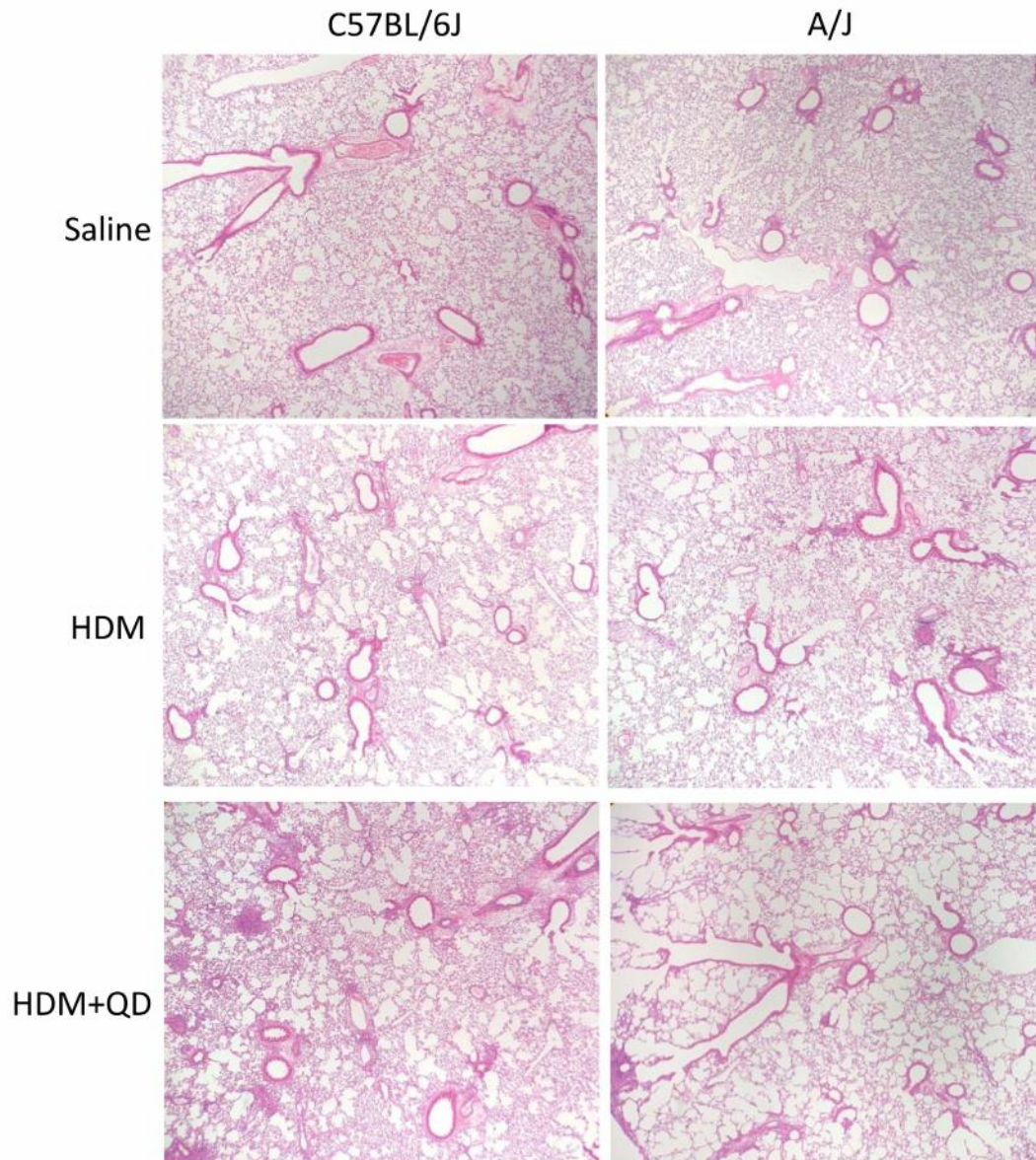


Figure S2. Representative images at 4x magnification of eosin and hematoxylin stained sections from a representative mouse from each treatment group from both C57BL/6J and A/J mice. Saline treated C57BL6/J mice (top left panel) and A/J mice (top right panel) show minimal inflammation, primarily increased numbers of macrophages in the alveolar spaces. HDM treated C57BL/6J mice (middle left panel) and HDM treated A/J mice (middle right panel), and HDM+QD treated A/J mice (lower right panel) show mild to moderate multifocal perivascular edema, alveolitis and interstitial pneumonia, and multinucleated giant cells in the alveolar spaces often associate with the alveolitis and interstitial

pneumonia. There is also mild to moderate multifocal perivascular cuffing with polymorphonuclear cells, histocytes and lymphocytes. C57BL/6J mice treated with HDM+QD (lower left panel) show lesions consistent to those seen with those seen in C57BL/6J and A/J mice treated with HDM, and A/J mice treated with HDM+QD, but the lesions tend to be moderate to severe.